

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
2 August 2001 (02.08.2001)

PCT

(10) International Publication Number  
**WO 01/55347 A1**

(51) International Patent Classification<sup>7</sup>: C12N 5/04,  
15/09, 15/29, 15/31, 15/82, A01H 5/00

(21) International Application Number: PCT/US01/02579

(22) International Filing Date: 26 January 2001 (26.01.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/178,565 26 January 2000 (26.01.2000) US

(71) Applicant: CORNELL RESEARCH FOUNDATION,  
INC. [US/US]; Suite 105, 20 Thornwood Drive, Ithaca, NY  
14850 (US).

(72) Inventors: BEER, Steven, V.; 211 Hudson Street, Ithaca,  
NY 14850 (US). BAUER, David, W.; 10815 115th Court  
NE, Kirkland, WA 98033 (US).

(74) Agents: GOLDMAN, Michael, L. et al.; Nixon Peabody  
LLP, Clinton Square, P.O. Box 31051, Rochester, NY  
14603-1051 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,  
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report
- before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: OOMYCETE-RESISTANT TRANSGENIC PLANTS BY VIRTUE OF PATHOGEN-INDUCED EXPRESSION OF A  
HETEROLOGOUS HYPERSENSITIVE RESPONSE ELICITOR

(57) Abstract: The present invention relates to a chimeric gene that includes a first DNA molecule encoding a hypersensitive re-  
sponse elicitor protein or polypeptide, a promoter operably linked 5' to the first DNA molecule to induce transcription of the first  
DNA molecule in response to activation of the promoter by an oomycete, and a 3' regulatory region operably linked to the first DNA  
molecule. Also disclosed are an expression system and a host cell containing the chimeric gene. The present invention also relates to  
a transgenic plant resistant to disease resulting from oomycete infection, the transgenic plant including the chimeric gene, wherein  
the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete. Transgenic seeds  
and transgenic cultivars obtained from the transgenic plant are also disclosed. Additional aspects of the present invention include  
methods of making a recombinant plant cell and a transgenic plant.

WO 01/55347 A1

**THIS PAGE BLANK (USPTO)**

**OOMYCETE-RESISTANT TRANSGENIC PLANTS BY VIRTUE OF  
PATHOGEN-INDUCED EXPRESSION OF A HETEROLOGOUS  
HYPERSENSITIVE RESPONSE ELICITOR**

5                   This application claims the benefit of U.S. Provisional Patent  
Application Serial No. 60/178,565, filed January 26, 2000, which is hereby  
incorporated by reference in its entirety.

                  This invention was made in part with support by the U.S. Government  
under Grant No. 97-34367-3937 from the U.S. Department of Agriculture. The U.S.  
10   Government may have certain rights in this invention.

**FIELD OF THE INVENTION**

                  The present invention relates to transgenic plants resistant to oomycete  
15   infection which contain a heterologous hypersensitive response elicitor under the  
control of a promoter responsive to infection by an oomycete.

**BACKGROUND OF THE INVENTION**

20                   In general, fungal plant diseases can be classified into two types: those  
caused by soilborne fungi and those caused by airborne fungi. Soilborne fungi cause  
some of the most widespread and serious plant diseases, such as root and stem rot  
caused by *Fusarium spp.* and root rot caused by *Phytophthora spp.* For example,  
*Phytophthora parasitica* var. *nicotiana*, a soilborne oomycete found in many tobacco  
25   growing regions worldwide, causes black shank, a highly destructive root and stem rot  
disease of many varieties of cultivated tobacco.

                  Since airborne fungi can be spread long distances by wind, they can  
cause devastating losses, particularly in crops which are grown over large regions. A  
number of pathogens have caused widespread epidemics in a variety of crops.  
30   Important diseases caused by airborne fungi are stem rust (*Puccinia graminis*) on  
wheat, corn smut (*Ustilago maydis*) on corn, and late blight disease (*Phytophthora*  
*infestans*) on potato and tomato. *Plasmopera viticola* is an airborne oomycete that  
causes downy mildew disease on grape vines. The blue mold fungus (*Peronospora*

*tabacina*) has caused catastrophic losses in tobacco crops, particularly in the United States and Cuba.

Most of these fungal diseases are difficult to combat, and farmers and growers must use a combination of practices, such as sanitary measures, resistant cultivars, and effective fungicide against such diseases. Hundreds of millions of dollars are spent annually for chemical control of plant-pathogenic fungi. As a result, there is today a real need for new, more effective and safe means to control plant-pathogenic fungi, particularly oomycetes which are responsible for major crop loss.

Genetic engineering promises to be an effective strategy for reducing the losses associated with diseases of field crops. Several successful approaches have been reported where the constitutive expression of antimicrobial peptides such as cecropins (Arce et al., "Enhanced Resistance to Bacterial Infection by *Erwinia Carotovora* Susp. *Atroseptica* in Transgenic Potato Plants Expressing the Attacin or the Cecropin SB-37 Genes," Am. J. Potato Res. 76:169-177 (1999)), lysozyme (Nakajima et al., "Fungal and Bacterial Disease Resistance in Transgenic Plants Expressing Human Lysozyme," Plant Cell Reports 16:674-679 (1997)), and monoclonal antibodies (Tavladoraki et al., "Transgenic Plants Expressing a Functional Single Chain FV Antibody are Specifically Protected from Virus Attack," Nature 366:468-472 (1993)) effectively protected plants from parasitic organisms. However, successful, these approaches have limited application to food production since many of these antimicrobial peptides and plant defense molecules are potentially toxic or allergenic to humans (Franck-Oberaspach et al., "Consequences of Classical and Biotechnological Resistance Breeding for Food Toxicology and Allergenicity," Plant Breeding 116:1-17 (1997)). Thus, alternative approaches for genetically engineering disease resistance would be more desirable.

Plants possess a highly evolved pathogen surveillance system which allows for recognition of specific pathogen derived molecules known as elicitors. Elicitor recognition results in an incompatible plant-microbe interaction, defined as the rapid activation of plant defense genes, typically resulting in the hypersensitive response and the onset of systemic acquired resistance.

The hypersensitive response is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z.,



“Defenses Triggered by the Invader: Hypersensitivity,” pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., “Hypersensitivity,” pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ( $\geq 10^7$  cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum) (Klement, Z., “Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads,” Nature 199:299-300; Klement, et al., “Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf,” Phytopathology 54:474-477 (1963); Turner, et al., “The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction,” Phytopathology 64:885-890 (1974); Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, (1982), these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., “Gene Cluster of *Pseudomonas syringae* pv. ‘phaseolicola’ Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants,” J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., “*hrp* Genes of Phytopathogenic Bacteria,” pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants

- and Animals - Molecular and Cellular Mechanisms, J.L. Dangel, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "Pseudomonas Syringae pv. Syringae Harpin<sub>Pss</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993); Wei, Z.-M., et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M., et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

- The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 *PopA1* protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al., "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

- Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "Erwinia chrysanthemi Harpin<sub>Ech</sub>: Soft-Rot Pathogenesis," MPMI 8(4): 484-91

(1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA<sup>-</sup> Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

Because the hypersensitive response results in localized necrosis of plant tissue, it is desirable to limit expression of a heterologous hypersensitive response elicitor to certain tissues in transgenic plants. This approach is discussed generally in PCT publication WO 94/01546 to Beer et al., but no specific transgenic plants are identified and only two suitable fungus-responsive promoters are suggested, e.g., the phenylalanine ammonia lyase and chalcone synthase promoters. No promoters responsive specifically to infection by oomycetes are identified therein.

The present invention is directed to overcoming these and other deficiencies in the art.

## SUMMARY OF THE INVENTION

20

The present invention relates to a chimeric gene that includes a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and a 3' regulatory region operably linked to the first DNA molecule. Also disclosed are an expression system that includes a vector in which is inserted a chimeric gene of the present invention and a host cell that includes a chimeric gene of the present invention.

Another aspect of the present invention relates to a transgenic plant resistant to disease resulting from oomycete infection. The transgenic plant includes a chimeric gene of the present invention, wherein the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete.

Transgenic seeds and transgenic cultivars obtained from the transgenic plant are also disclosed.

5 An additional aspect of the present invention relates to a method of making a recombinant plant cell. This is accomplished by transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter.

10 A further aspect of the present invention relates to a method of making a plant resistant to disease resulting from oomycete infection. This is accomplished by transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter and regenerating the plant from the transformed plant cell.

15 The present invention confers oomycete-induced disease resistance to plants transformed with a chimeric gene encoding a hypersensitive response elicitor protein or polypeptide, which is transcribed within a limited population of plant cells in response to infection of the plant by an oomycete. To limit transcription of the chimeric gene within a certain population of plant cells, the chimeric gene includes a promoter that is responsive to infection by an oomycete (i.e., it is activated by the oomycete). The hypersensitive response elicitor protein or polypeptide can cause tissue collapse at the site of infection and/or induce systemic resistance against the oomycete and other pathogens. By using the promoter from the potato *gst1* gene, for example, which is activated by infection with oomyceteous fungi, the present invention can control fungal pathogens within crops without harming the transgenic plant and without resorting to use of environmentally damaging chemicals.

20  
25

### BRIEF DESCRIPTION OF THE DRAWINGS

30 Figure 1 is a schematic representation and partial restriction map of T-DNA in plant transformation vector pCPP1294. Filled triangles represent the left and right borders; *Pgst1* represents the *gst1* promoter from potato variety Atlantic; PR1-b represents the DNA molecule encoding a signal sequence from *Nicotiana tabacum*;

*hrpN* represents the DNA molecule encoding the hypersensitive response elicitor harpin<sub>Ea</sub> of *Erwinia amylovora*; NT represents the nos terminating region; *aacC1* represents the gentamycin resistance cassette.

Figure 2 is an image of transgenic *Arabidopsis* plants containing a construct encoding GUS under control of the *gst1* promoter. To demonstrate pathogen inducibility of the *gst1* promoter in *Arabidopsis*, GUS staining was measured following inoculation of the plants with water (left) or *P. parasitica* (right). GUS expression is indicated by dark staining.

Figures 3A and 3B show an analysis of *hrpN* gene expression in *Arabidopsis* transgenic-line GSSN8-4, containing the construct shown in Figure 1, after inoculation with *P. parasitica* NOCO. At one day intervals leaves were collected for isolation of total RNA. Figure 3A is a Northern blot analysis performed using *hrpN* DNA as a probe. Figure 3B is an ethidium bromide stained gel shown as a control (bottom).

Figures 4A and 4B are images demonstrating *Arabidopsis* GSSN 8-4 are resistant to *P. parasitica*. Figure 4A shows the effects of *P. parasitica* infection in WT *Arabidopsis* (control, left) and GSSN 8-4 *Arabidopsis* (test, right). Figure 4B shows the degree of trypan blue staining of *P. parasitica*-infected leaves of WT (control, left) and GSSN 8-4 plants (test, right), both taken 10 days post-inoculation.

Figure 5 is a graph depicting the severity of *P. parasitica* infection in WT (control), EV (control), and *hrpN* transgenic plants (test). Two week old plants were drop inoculated with conidiospores of *P. parasitica* (2 ml drops;  $5 \times 10^4$  spores/ml). Ten days after inoculation, 30 plants of each genotype were rated for disease severity. Ratings were adapted from Cao et al. ("Generation of Broad-Spectrum Disease Resistance by Overexpression of an Essential Regulatory Gene in Systemic Acquired Resistance," Proc. Natl. Acad. Sci. USA 95:6531-6536 (1998), which is hereby incorporated by reference) as follows: 1, no conidiophores present on plant; 2, 0-5 conidiophores per infected plant; 3, 6-20 conidiophores present on a few infected leaves; 4, 6-20 conidiophores present on most infected leaves; 5, more than 20 conidiophores on all infected leaves.

## DETAILED DESCRIPTION OF THE INVENTION

One aspect of the present invention relates to a novel DNA construct in the form of a chimeric gene. The chimeric gene includes a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and a 3' regulatory region operably linked to the first DNA molecule. As discussed more fully hereinafter, a chimeric gene of the present invention is particularly useful in preparing a transgenic plant for the purpose of rendering the transgenic plant resistant to disease resulting from infection thereof by an oomycete.

The first DNA molecule can encode any hypersensitive response elicitor protein or polypeptide which is effective in triggering a hypersensitive response (i.e., in a particular host plant selected for transformation). Generally, it is desirable to express hypersensitive response elicitors only in plants which are non-hosts for the source organism of the hypersensitive response elicitor. Suitable hypersensitive elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

Exemplary hypersensitive response elicitor proteins and polypeptides from bacterial sources include, without limitation, the hypersensitive response elicitors from *Erwinia* species (e.g., *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, etc.), *Pseudomonas* species (e.g., *Pseudomonas syringae*, *Pseudomonas solanacearum*, etc.), and *Xanthomonas* species (e.g., *Xanthomonas campestris*). In addition to hypersensitive response elicitors from these Gram-negative bacteria, it is possible to use elicitors from Gram-positive bacteria. One example is the hypersensitive response elicitor from *Clavibacter michiganensis* subsp. *sepedonicus*.

Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors (i.e., elicitors) from various *Phytophthora* species (e.g., *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, *Phytophthora citrophthora*, etc.).

- 9 -

Preferably, the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide of *Erwinia chrysanthemi*, *Erwinia amylovora*, *Pseudomonas syringae*, or *Pseudomonas solanacearum*.

The hypersensitive response elicitor protein or polypeptide from  
 5 *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1  
 as follows:

	Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser	
	1				5					10					15		
10	Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser	
				20					25					30			
	Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr	
			35					40					45				
15	Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu	
		50					55					60					
	Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser	
	65				70					75						80	
	Phe	Gly	Asn	Gly	Ala	Gln	Gly	Ala	Ser	Asn	Leu	Leu	Ser	Val	Pro	Lys	
				85						90					95		
20	Ser	Gly	Gly	Asp	Ala	Leu	Ser	Lys	Met	Phe	Asp	Lys	Ala	Leu	Asp	Asp	
				100					105					110			
	Leu	Leu	Gly	His	Asp	Thr	Val	Thr	Lys	Leu	Thr	Asn	Gln	Ser	Asn	Gln	
			115					120					125				
25	Leu	Ala	Asn	Ser	Met	Leu	Asn	Ala	Ser	Gln	Met	Thr	Gln	Gly	Asn	Met	
		130					135					140					
	Asn	Ala	Phe	Gly	Ser	Gly	Val	Asn	Asn	Ala	Leu	Ser	Ser	Ile	Leu	Gly	
	145					150				155						160	
	Asn	Gly	Leu	Gly	Gln	Ser	Met	Ser	Gly	Phe	Ser	Gln	Pro	Ser	Leu	Gly	
				165					170						175		
30	Ala	Gly	Gly	Leu	Gln	Gly	Leu	Ser	Gly	Ala	Gly	Ala	Phe	Asn	Gln	Leu	
				180					185					190			
	Gly	Asn	Ala	Ile	Gly	Met	Gly	Val	Gly	Gln	Asn	Ala	Ala	Leu	Ser	Ala	
			195					200					205				
35	Leu	Ser	Asn	Val	Ser	Thr	His	Val	Asp	Gly	Asn	Asn	Arg	His	Phe	Val	
		210					215					220					
	Asp	Lys	Glu	Asp	Arg	Gly	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met	Asp	
	225					230				235						240	

- 10 -

	Gln Tyr Pro Glu Ile Phe Gly Lys	Pro Glu Tyr Gln Lys Asp Gly Trp	
	245	250	255
	Ser Ser Pro Lys Thr Asp Asp Lys Ser	Trp Ala Lys Ala Leu Ser Lys	
	260	265	270
5	Pro Asp Asp Asp Gly Met Thr Gly Ala	Ser Met Asp Lys Phe Arg Gln	
	275	280	285
	Ala Met Gly Met Ile Lys Ser Ala Val	Ala Gly Asp Thr Gly Asn Thr	
	290	295	300
10	Asn Leu Asn Leu Arg Gly Ala Gly Gly	Ala Ser Leu Gly Ile Asp Ala	
	305	310	315
	Ala Val Val Gly Asp Lys Ile Ala Asn	Met Ser Leu Gly Lys Leu Ala	
	325	330	335
	Asn Ala		

15

This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. This *Erwinia chrysanthemi* hypersensitive response elicitor protein or polypeptide is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

20

25	cgatttttacc	cgggtgaacg	tgctatgacc	gacagcatca	cggatttcga	caccgttacg	60
	gcgtttatgg	ccgcgatgaa	ccggcatcag	gcggcgcgct	ggtcgccgca	atccggcgctc	120
	gatctgggtat	ttcagtttg	ggacaccggg	cgtgaactca	tgatgcagat	tcagccgggg	180
	cagcaatatc	ccggcatgtt	gcgcacgctg	ctcgctcgtc	gttatcagca	ggcggcagag	240
	tgcgatggct	gccatctgtg	cctgaacggc	agcgatgtat	tgatcctctg	gtggccgctg	300
	ccgtcggatc	ccggcagtta	tccgcagggtg	atcgaacggt	tgtttgaact	ggcgggaatg	360
30	acgttgccgt	cgctatccat	agcaccgacg	gcgcgtccgc	agacagggaa	cggacgcgcc	420
	cgatcattaa	gataaaggcg	gcttttttta	ttgcaaaacg	gtaacgggtga	ggaaccgttt	480
	caccgctcggc	gtcactcagt	aacaagtatc	catcatgatg	cctacatcgg	gatcggcgctg	540
	ggcatccgtt	gcagatactt	ttgcgaacac	ctgacatgaa	tgaggaaacg	aaattatgca	600
	aattacgata	aaagcgcaca	tcggcggtga	tttgggcgctc	tccgggtctgg	ggctgggtg	660
	tcagggactg	aaaggactga	attccgcggc	ttcatcgctg	ggttcacagc	tggataaact	720
35	gagcagcacc	atcgataagt	tgacctccgc	gctgacttcg	atgatgtttg	gcggcgcgct	780
	ggcgcagggg	ctgggcgcca	gctcgaagg	gctggggatg	agcaatcaac	tgggccagtc	840
	tttcggcaat	ggcgcgcagg	gtgcgagcaa	cctgctatcc	gtaccgaaat	ccggcgcgca	900



- 11 -

5 tcggttgatca aaaatgtttg ataaagcgtt ggacgatctg ctgggtcatg acaccgtgac 960  
 caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020  
 ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcactgtcgt ccatttctcg 1080  
 caacggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggctt 1140  
 gcagggcctg agcgggcgcg gtgcattcaa ccagttgggt aatgccatcg gcatgggcgt 1200  
 ggggcagaat gctgcgctga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260  
 ccgccacttt gtagataaag aagatcgcg catggcgaaa gagatcgggc agtttatgga 1320  
 tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380  
 gacggacgac aaatcctggg ctaaagcgtt gagtaaaccg gatgatgacg gtatgaccgg 1440  
 10 cgccagcatg gacaaattcc gtcaggcgat gggatatgatc aaaagcggcg tggcgggtga 1500  
 taccggcaat accaacctga acctgcgtgg cgcggggcgt gcatcgctgg gtatcgatgc 1560  
 ggctgtcgtc ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620  
 atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680  
 ttattatgcg gtttatgcgg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740  
 15 acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttctc 1800  
 gtcgctcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc 1860  
 cagatggaga cagctctgcg ataaatctgt gccgtaacgt gtttctatcc gccccttag 1920  
 cagatagatt gcggtttcgt aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980  
 gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040  
 20 aaaatagggc agtttttgcg tggatccgt ggggtgttcc ggctgacaa tcttgagttg 2100  
 gttcgtcatc atctttctcc atctgggcga cctgatcggt t 2141

The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID.

25 No. 3 as follows:

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
 1 5 10 15  
 30 Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
 20 25 30  
 Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
 35 40 45  
 Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
 50 55 60

- 12 -

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
 65 70 75 80  
 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
 85 90 95  
 5 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
 100 105 110  
 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
 115 120 125  
 10 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
 130 135 140  
 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
 145 150 155 160  
 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
 165 170 175  
 15 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu  
 180 185 190  
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly  
 195 200 205  
 20 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly  
 210 215 220  
 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu  
 225 230 235 240  
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln  
 245 250 255  
 25 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln  
 260 265 270  
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe  
 275 280 285  
 30 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met  
 290 295 300  
 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro  
 305 310 315 320  
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser  
 325 330 335  
 35 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn  
 340 345 350  
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
 355 360 365

- 13 -

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp  
 370 375 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu  
 385 390 395 400

5 Gly Ala Ala

This hypersensitive response elicitor protein or polypeptide has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor protein or polypeptide has substantially  
 10 no cysteine. The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA  
 15 molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa 60  
 gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct 120  
 atcggcgggtg cgggcggaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg 180  
 20 ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg 240  
 gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcgggtg tgggctgatg 300  
 ggcggtggct taggcgggtg cttaggtaat ggcttgggtg gctcaggtgg cctgggcgaa 360  
 ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa 420  
 ggcggcaaca ataccacttc aacaacaaat tccccgctgg accaggcgct gggatttaac 480  
 25 tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac 540  
 ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggatgatggg 600  
 caagatggca cccagggcag ttccctctggg ggcaagcagc cgaccgaagg cgagcagaac 660  
 gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag 720  
 ctcccttgga acgggggact gggaggtggt cagggcggtg atgctggcac gggctctgac 780  
 30 ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccgggtgga ctaccagcag 840  
 ttaggtaacg ccgtgggtac cggtatcggt atgaaagcgg gcattcaggc gctgaatgat 900  
 atcgggtacgc acaggcacag ttcaaccctg tctttcgtca ataaaggcga tcgggcgatg 960  
 gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac 1020  
 cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc 1080

- 14 -

aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc 1140  
 atgatcaaaa ggcccatggc gggtgatacc ggcaacggca acctgcaggc acgcggtgcc 1200  
 ggtgggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca 1260  
 cttggcaagc tgggcgcggc ttaagctt 1288

5

The hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

10 Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
 1 5 10 15  
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser  
 20 25 30  
 15 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met  
 35 40 45  
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala  
 50 55 60  
 20 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val  
 65 70 75 80  
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
 85 90 95  
 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met  
 100 105 110  
 25 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
 115 120 125  
 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
 130 135 140  
 30 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
 145 150 155 160  
 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
 165 170 175  
 Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
 180 185 190  
 35 Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
 195 200 205  
 Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
 210 215 220

- 15 -

	Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser	
	225					230					235					240	
	Gly	Asn	Thr	Arg	Gly	Glu	Ala	Gly	Gln	Leu	Ile	Gly	Glu	Leu	Ile	Asp	
					245					250					255		
5	Arg	Gly	Leu	Gln	Ser	Val	Leu	Ala	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Val	
				260					265					270			
	Asn	Thr	Pro	Gln	Thr	Gly	Thr	Ser	Ala	Asn	Gly	Gly	Gln	Ser	Ala	Gln	
			275					280					285				
10	Asp	Leu	Asp	Gln	Leu	Leu	Gly	Gly	Leu	Leu	Leu	Lys	Gly	Leu	Glu	Ala	
	290						295					300					
	Thr	Leu	Lys	Asp	Ala	Gly	Gln	Thr	Gly	Thr	Asp	Val	Gln	Ser	Ser	Ala	
	305					310					315					320	
	Ala	Gln	Ile	Ala	Thr	Leu	Leu	Val	Ser	Thr	Leu	Leu	Gln	Gly	Thr	Arg	
					325					330					335		
15	Asn	Gln	Ala	Ala	Ala												
					340												

This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

- 20 Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., et al., "*Pseudomonas syringae* pv. *syringae* Harpin<sub>PS</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding this hypersensitive response
- 25 elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

	atgcagagtc	tcagtccttaa	cagcagctcg	ctgcaaacc	cggcaatggc	ccttgctctg	60
	gtacgtcctg	aagccgagac	gactggcagt	acgtcgagca	aggcgcttca	ggaagttgtc	120
30	gtgaagctgg	ccgaggaact	gatgcgcaat	ggtcaactcg	acgacagctc	gccattggga	180
	aaactgttgg	ccaagtcgat	ggccgcagat	ggcaaggcgg	gcggcggtat	tgaggatgtc	240
	atcgctgcgc	tggacaagct	gatccatgaa	aagctcggtg	acaacttcgg	cgcgtctgcg	300
	gacagcgctt	cgggtaccgg	acagcaggac	ctgatgactc	aggtgctcaa	tggcctggcc	360
	aagtcgatgc	tcgatgatct	tctgaccaag	caggatggcg	ggacaagctt	ctccgaagac	420
35	gatatgccga	tgctgaacaa	gatcgcgag	ttcatggatg	acaatcccg	acagtttccc	480
	aagccggact	cgggctcctg	ggtgaacgaa	ctcaaggaag	acaacttcct	tgatggcgac	540

5 gaaacggctg cggtccgttc ggcaactcgac atcattggcc agcaactggg taatcagcag 600  
 agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcactccgag cagtttttcc 660  
 aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc 720  
 ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780  
 tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgagac cggtagctcg 840  
 gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggcctt gctgctcaag 900  
 ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960  
 gcgcaaactg ccaccttgct ggtcagtagc ctgctgcaag gcacccgcaa tcaggctgca 1020  
 gcctga 1026

10

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference.

15 The hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln 15  
 1 5 10  
 20 Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser 30  
 20 20 25 30  
 Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile 45  
 35 40 45  
 25 Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly 60  
 50 55 60  
 Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala 80  
 65 70 75 80  
 Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser 95  
 85 90 95  
 30 Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met 110  
 100 105 110  
 Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala 125  
 115 120 125  
 35 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val 140  
 130 135 140  
 Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala 160  
 145 150 155 160

- 17 -

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
 165 170 175  
 Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
 180 185 190  
 5 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
 195 200 205  
 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
 210 215 220  
 10 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240  
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
 245 250 255  
 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
 260 265 270  
 15 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
 275 280 285  
 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
 290 295 300  
 20 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
 305 310 315 320  
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
 325 330 335  
 Gln Ser Thr Ser Thr Gln Pro Met  
 340

25

Further information regarding this hypersensitive response elicitor protein or  
 polypeptide derived from *Pseudomonas solanacearum* is set forth in Arlat, M., et al.,  
 "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia  
 Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO  
 30 J. 13:543-533 (1994), which is hereby incorporated by reference. It is encoded by a  
 DNA molecule from *Pseudomonas solanacearum* having a nucleotide sequence  
 corresponding SEQ. ID. No. 8 as follows:

atgtcagtcg gaaacatcca gagcccgctcg aacctcccgg gtctgcagaa cctgaacctc 60  
 35 aacaccaaca ccaacagcca gcaatcgggc cagtccgtgc aagacctgat caagcaggtc 120  
 gagaaggaca tcctcaacat catcgagcc ctcgtgcaga aggccgcaca gtcggcgggc 180  
 ggcaacaccg gtaacaccgg caacgcgccg gcgaaggacg gcaatgcca cgcggggcgcc 240

- 18 -

aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc 300  
 ggcaacgtcg acgacgcaa caaccaggat ccgatgcaag cgctgatgca gctgctggaa 360  
 gacctggtga agctgctgaa ggcgccctg cacatgcagc agcccggcgg caatgacaag 420  
 ggcaacggcg tgggcggtgc caacggcgcc aaggggtgccg gcggccaggg cggcctggcc 480  
 5 gaagcgctgc aggagatcga gcagatcctc gcccagctcg gcggcggcgg tgctggcgcc 540  
 ggcggcgcgg gtggcggtgt cggcggtgct ggtggcgcg atggcggtc cggcgcggtt 600  
 ggcgcgaggc gtgcgaacgg cgcgcagcgg ggcaatggcg tgaacggcaa ccaggcgaac 660  
 ggcccgcaga acgcaggcga tgtcaacggg gccaacggcg cggatgacgg cagcgaagac 720  
 caggcgcgcc tcaccggcgt gctgcaaaag ctgatgaaga tctgaacgc gctggtgcag 780  
 10 atgatgcagc aaggcgccct cggcggcggc aaccaggcgc agggcggtc gaaggggtgcc 840  
 ggcaacgcct cgcgggcttc cggcgcgaa cggggcgga accagcccgg ttcggcggat 900  
 gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc 960  
 gtccagatcc tgcagcagat gctggcgggc cagaacggcg gcagccagca gtccacctc 1020  
 acgcagccga tgtaa 1035

15

Other embodiments of the present invention include, but are not limited to, use of the nucleotide sequence encoding for the hypersensitive response elicitor protein or polypeptide from *Erwinia carotovora* and *Erwinia stewartii*. Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

The hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et al., "Structure and Activity of Proteins from Pathogenic Fungi



Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the  
5 Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet. et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants." Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

10 Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

Other elicitors can be readily identified by isolating putative  
15 hypersensitive response elicitors and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to  
20 infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using standard techniques known to those skilled in the art. The isolated DNA molecule can then be introduced into the chimeric gene for expression in a transgenic plant of the present invention.

The first DNA molecule can also encode fragments of the above  
25 hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens.

Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by  
30 Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding

editions), which are hereby incorporated by reference. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., Science 257: 85-88 (1992), which is hereby incorporated by reference.

- 5 In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference.
- 10 These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

- An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora* hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The
- 15 fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ.
- 20 ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.
- 25 DNA molecules encoding these fragments can also be utilized in the chimeric gene of the present invention.

- The first DNA molecule also can be a DNA molecule that hybridizes under stringent conditions to the DNA molecule having nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, or 8. An example of suitable stringency conditions is when
- 30 hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the

temperature for either hybridization or washing conditions or increasing the sodium concentration of the hybridization or wash medium. Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 µg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

Variants of suitable hypersensitive response elicitor proteins or polypeptides can also be expressed by the first DNA molecule. Variants may be made by, for example, the deletion, addition, or alteration of amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide (such as a 6xHis tag).

The promoter of the chimeric gene should be selected on the basis of its ability to induce transcription of the first DNA molecule in response to infection of the plant by an oomycete (i.e., the oomycete activates the promoter).

According to one embodiment, the promoter preferably includes some or all of the promoter-effective regions of a *gst1* gene from potato. The *gst1* promoter is activated in response to infection by oomycetes and not by wounding or other environmental perturbations. The *gst1* promoter from potato has a nucleic acid sequence corresponding to SEQ. ID. No. 9 as follows:

```

gaattcagga agaattttgt aggttcaact aaattatata tatatatata aaaaaataaa    60
aattattaga cgcttcgact atttacttac tttaaaattt gaattttcgt acgaataaaa    120
ttatttgtca gagaaaagtc ttttagctat tcacatgcta ggaagtttca cttttggtgg    180

```

- 22 -

atcagtgatt gtatattatt taatatatat caatthttctc atcaaactga aaatgaaaga 240  
 taaaattaat attaaaaact ccattcattt taattttattg tcatgttttg acttgatcca 300  
 aaatctaaca atttaaaagg ttttaaattht ttgtgttttt ttttaaattha aaaatatgtc 360  
 aaatatatta aaatatatttt ttttaaattht atactaaaaa acatgtcaca tgaatatthtg 420  
 5 aaattataaaa attatcaaaa ataaaaaaag aatattttctt taacaaatta aaattgaaaa 480  
 tatgataaat aaattaaact attctatcat tgattttttct agccaccaga tttgacccaaa 540  
 cagtgggtga catgagcaca taagtcattt ttattgtatt ttattactca ctccaaaaat 600  
 ataggaata tgthttactac ttaattttagt caaatataat ttttatattag aataattgaa 660  
 tagtcaaaaa agaaacttta atgcattctt atthttt 696

10

Effective fragments of SEQ. ID. No. 9 are also encompassed by the present invention. U.S. Patent Nos. 5,750.874 and 5,723.760 to Strittmayer et al., which are hereby incorporated by reference, define promoter-effective regions of the potato *gstI* promoter. Preferably, the *gstI* promoter includes a nucleotide sequence corresponding, at a minimum, to nucleotides 295-567 of SEQ. ID. No. 9. The *gstI* promoter can also include effective portions containing nucleotides 295-696 of SEQ. ID. No. 9.

The chimeric gene of the present invention also includes an operable 3' regulatory region, selected from among those which are capable of providing correct transcription termination and polyadenylation of mRNA for expression in plant cells, operably linked to the first DNA molecule which encodes for a hypersensitive response elicitor. A number of 3' regulatory regions are known to be operable in plants. Exemplary 3' regulatory regions include, without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference) and the cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference). Virtually any 3' regulatory region known to be operable in plants would suffice for proper expression of the coding sequence of the chimeric gene of the present invention.

The first DNA molecule, promoter, and a 3' regulatory region can be ligated together using well known molecular cloning techniques described in

Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference.

The chimeric gene can also include a second DNA molecule encoding a secretion signal. A number of suitable secretion signals are known in the art and other are continually being identified. The secretion signal can be an RNA leader which directs secretion of the subsequently transcribed protein or polypeptide, or the secretion signal can be an amino terminal peptide sequence that is recognized by a host plant secretory pathway. The second DNA molecule can be ligated between the promoter and the first DNA molecule, using known molecular cloning techniques as indicated above.

According to one embodiment, the second DNA molecule encodes a secretion signal derived from *Nicotiana tabacum*. Specifically, this DNA molecule encodes the secretion signal polypeptide for *PR1-b* gene of *Nicotiana tabacum*. This second DNA molecule has a nucleotide sequence corresponding to SEQ. ID. No. 10 as follows:

```
tctagaccat gggatttttt ctcttttcac aaatgccctc attttttctt gtgtcgacac 60
ttctcttatt cctaataata tctcactctt ctcatgccca aaactctaga 110
```

The above sequence includes XbaI sites (underlined) at each end to facilitate insertion of the second DNA molecule into the chimeric gene of the present invention. The coding sequence of SEQ. ID. No. 10 starts at base 8. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

```
Met Gly Phe Phe Leu Phe Ser Gln Met Pro Ser Phe Phe Leu Val Ser
  1           5           10           15
Thr Leu Leu Leu Phe Leu Ile Ile Ser His Ser Ser His Ala Gln Asn
30           20           25           30
Ser Arg
```

An alternative second DNA molecule encoding the secretion signal polypeptide for *PR1-b* gene of *Nicotiana tabacum* has a nucleotide sequence corresponding to SEQ. ID. No. 12 as follows:

- 24 -

atgggatttt ttctcttttc acaaatgcc tcattttttc ttgtctctac acttctctta 60  
 ttctaataa tatctcactc ttctcatgcc caaaactctc aa 102

- 5 This nucleotide sequence is disclosed in Genbank Accession No. X03465, which is hereby incorporated by reference. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 13 as follows:

10 Met Gly Phe Phe Leu Phe Ser Gln Met Pro Ser Phe Phe Leu Val Ser  
           1                  5                  10                  15  
 Thr Leu Leu Leu Phe Leu Ile Ile Ser His Ser Ser His Ala Gln Asn  
                   20                  25                  30  
 Ser Gln

- 15 Yet another second DNA molecule encodes the secretion signal for the *PR1-a* gene of *Nicotiana tabacum*. This DNA molecule has a nucleotide sequence corresponding to SEQ. ID. No. 14 as follows:

20 atgggatttg ttctcttttc acaattgect tcattttcttc ttgtctctac acttctctta 60  
 ttccatagtaa tatcccaactc ttgccgtgcc 90

- This DNA molecule is disclosed in Genbank Accession No. X06361, which is hereby  
 25 incorporated by reference. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 15 as follows:

30 Met Gly Phe Val Leu Phe Ser Gln Leu Pro Ser Phe Leu Leu Val Ser  
           1                  5                  10                  15  
 Thr Leu Leu Leu Phe Leu Val Ile Ser His Ser Cys Arg Ala  
                   20                  25                  30

- 35 Still another second DNA molecule encodes the secretion signal for the *PR4-a* gene of *Nicotiana tabacum*. This DNA molecule has a nucleotide sequence corresponding to SEQ. ID. No. 16 as follows:

atggagagag ttaataatta taagttgtgc gtggcattgt tgatcatcag catggtgatg 60  
 gcaatggcgg cggca 75

40

- 25 -

This DNA molecule is disclosed in Genbank Accession No. X58546, which is hereby incorporated by reference. The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

5           Met Glu Arg Val Asn Asn Tyr Lys Leu Cys Val Ala Leu Leu Ile Ile  
               1                               5                               10                               15  
              Ser Met Val Met Ala Met Ala Ala Ala  
    20                               25

10           Each second DNA molecule can be cloned using primers that introduce restriction sites at the 5' and 3' ends thereof to facilitate insertion of the second DNA molecule into the chimeric gene of the present invention. SEQ. ID. No. 10 is shown to include such restriction sites (e.g., XbaI).

              Further aspects of the present invention include an expression system  
 15   that includes a vector containing a chimeric gene of the present invention, as well as a host cell which includes a chimeric gene of the present invention. As described more fully hereinafter, the recombinant host cell can be either a bacterial cell (i.e., *Agrobacterium*) or a plant cell. In the case of recombinant plant cells, it is preferable that the chimeric gene is stably inserted into the genome of the recombinant plant cell.

20           The chimeric gene can be incorporated into cells using conventional recombinant DNA technology. Generally, this involves inserting the chimeric gene into an expression vector or system to which it is heterologous (i.e., not normally present). As described above, the chimeric gene contains the necessary elements for the transcription and translation in plant cells of the first DNA molecule (i.e.,  
 25   encoding the hypersensitive response elicitor protein or polypeptide) and, if present, the second DNA molecule.

              U.S. Patent No. 4,237,224 issued to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA  
 30   ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

              Once the chimeric gene of the present invention has been prepared, it is ready to be incorporated into a host cell. Recombinant molecules can be introduced

into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like. Preferably the host cells are either a bacterial cell or a plant cell.

Accordingly, another aspect of the present invention relates to a method of making a recombinant plant cell. Basically, this method is carried out by transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter. Preferably, the chimeric gene is stably inserted into the genome of the recombinant plant cell as a result of the transformation.

A related aspect of the present invention concerns a method of making a plant resistant to disease resulting from oomycete infection. Basically, this method is carried out by transforming a plant cell with a chimeric gene of the present invention under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter and regenerating a plant from the transformed plant cell.

One approach to transforming plant cells with a chimeric gene of the present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector



- 27 -

and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Another method of introducing the chimeric gene is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the chimeric gene. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The chimeric gene may also be introduced into the plant cells by electroporation. Fromm, et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the chimeric gene. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the chimeric gene into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with the chimeric gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

*Agrobacterium* is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences such as a chimeric gene of the present invention can be introduced into appropriate plant cells by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into

- 28 -

the plant genome. Schell, J., Science, 237:1176-83 (1987), which is hereby incorporated by reference.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

5 After transformation, the transformed plant cells can be selected and regenerated.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the chimeric gene of the present invention. Suitable selection markers include, without limitation, markers  
10 coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present  
15 invention. Cells or tissues are grown on a selection media containing an antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a full-grown plant therefrom. Thus, another aspect of the present  
20 invention relates to a transgenic plant that is resistant to disease resulting from oomycete infection. The transgenic plant includes a chimeric gene of the present invention, wherein the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete. Preferably, the chimeric gene is stably inserted into the genome of the transgenic plant of the present invention.

25 Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants. Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

30 It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce,

endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

5                   Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form  
10 plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and  
15 repeatable.

                  After the chimeric gene is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord  
20 with common agricultural procedures known to those in the field.

                  Resistance against different types of oomycetes may be imparted to transgenic plants according to the present invention. Without being bound by any particular theory, it is believed that a hypersensitive response elicitor protein or polypeptide encoded by the first DNA molecule is transcribed in response to infection  
25 of the plant by an oomycete. The exact mechanism by which the promoter is activated to regulate transcription of sequences under its control is not fully understood; however, the first DNA molecule is transcribed and the hypersensitive response elicitor is expressed in a limited population of cells (i.e., those in which transcription has been induced following oomycete infection). Once expressed, it is  
30 believed that the hypersensitive response elicitor can either be secreted from the plant cell (assuming the chimeric gene also contains a second DNA molecule encoding an N-terminal secretion signal) or leaked from an oomycete-infected plant cell.

- 30 -

Regardless of how the hypersensitive response elicitor is delivered to the intercellular environment, it is believed that the hypersensitive response elicitor protein or polypeptide will initiate a hypersensitive response to cause localized necrosis of oomycete-infected tissues. In addition, systemic acquired resistance may be developed by the transgenic plant following initiation of the hypersensitive response. This may yield broad disease and/or pathogen resistance to the transgenic plants of the present invention.

Oomycetes against which resistance is imparted include, without limitation, species of *Plasmopara*, *Phytophthora*, *Peronospora*, *Pseudoperonospora*, *Bremia*, *Sclerospora*, *Aphanomyces*, *Pythium*, and *Albugo*.

According to one embodiment of the present invention, an oomycete resistant transgenic tobacco plant includes a chimeric gene of the present invention, wherein expression of the encoded hypersensitive response elicitor is responsive to infection of the plant by an oomycete that is a pathogen of tobacco, including, but not limited to, *Peronospora tabacina* (which causes blue mold) and *Phytophthora parasitica* (which causes black shank).

The chimeric gene of the present invention can be utilized to impart oomycete resistance for a wide variety of tobacco plants, some of which may possess varying levels of natural resistance against pathogenic oomycetes. The varieties of tobacco plants which can be protected include, without limitation, those referred to as Coker 371 Gold, K 149, K 326, K 346, K 394, K 730, RG 11, RG17, RG22, Speight G-70, Speight G-117, Speight G-126, GL939, NC 55, NC 71, NC 72, NC 95, NC 2326, OX 207, OX 940, RG 81, RG H4, RG H61, Speight 168, Speight NF3, Speight 172, CU 236, CU 387, CU 368, NC TG91, OX 4142NF, OX 4083, RG 4H2-12, RG 4H2-17, RG 4H2-20, Speight 177, Speight 178, Speight 179, VPI 107, VPI 605, NG TG94, KY 14, KY 8959, KY 907, KY 908, TN 86, TN 90, TN 97, VA 116, VA 509, B 21 x KY 10, KY 14 x L8, NC 3, NC BH129, DH332, COOP 313, COOP 543, Clay's 403, Clay's 502, HY 402, PF 561, and R 711.

According to another embodiment of the present invention, an oomycete resistant transgenic grape plant includes a chimeric gene of the present invention, wherein expression of the encoded hypersensitive response elicitor is responsive to infection of the plant by an oomycete that is a pathogen of grape,

including, but not limited to, *Plasmopara viticola* (which causes downy mildew), *Pythium* spp. (which cause root and/or stem rot), and *Phytophthora* spp. (which cause root and/or stem rot).

The chimeric gene of the present invention can be utilized to impart oomycete resistance for a wide variety of grapevine plants. The chimeric gene is particularly well suited to imparting resistance to *Vitis* scion or rootstock cultivars. Scion cultivars which can be protected include, without limitation, those commonly referred to as Table or Raisin Grapes, such as Alden, Almeria, Anab-E-Shahi, Autumn Black, Beauty Seedless, Black Cornish, Black Damascus, Black Malvoisie, Black Prince, Blackrose, Bronx Seedless, Burgrave, Calmeria, Campbell Early, Canner, Cardinal, Catawba, Christmas, Concord, Dattier, Delight, Diamond, Dizmar, Duchess, Early Muscat, Emerald Seedless, Emperor, Exotic, Ferdinand de Lesseps, Fiesta, Flame seedless, Flame Tokay, Gasconade, Gold, Himrod, Hunisa, Hussiene, Isabella, Italia, July Muscat, Khandahar, Katta, Kourgane, Kishmishi, Loose Perlette, Malaga, Monukka, Muscat of Alexandria, Muscat Flame, Muscat Hamburg, New York Muscat, Niabell, Niagara, Olivette blanche, Ontario, Pierce, Queen, Red Malaga, Ribier, Rish Baba, Romulus, Ruby Seedless, Schuyler, Seneca, Suavis (IP 365), Thompson seedless, and Thomuscat. They also include, without limitation, those used in wine production, such as Aleatico, Alicante Bouschet, Aligote, Alvarelhao, Aramon, Baco blanc (22A), Burger, Cabernet franc, Cabernet, Sauvignon, Calzin, Carignane, Charbono, Chardonnay, Chasselas dore, Chenin blanc, Clairette blanche, Early Burgundy, Emerald Riesling, Feher Szagos, Fernao Pires, Flora, French Colombard, Fresia, Furmint, Gamay, Gewurztraminer, Grand noir, Gray Riesling, Green Hungarian, Green Veltliner, Grenache, Grillo, Helena, Inzolia, Lagrein, Lambrusco de Salamino, Malbec, Malvasia bianca, Mataro, Melon, Merlot, Meunier, Mission, Montua de Pilas, Muscadelle du Bordelais, Muscat blanc, Muscat Ottonel, Muscat Saint-Vallier, Nebbiolo, Nebbiolo fino, Nebbiolo Lampia, Orange Muscat, Palomino, Pedro Ximenes, Petit Bouschet, Petite Sirah, Peverella, Pinot noir, Pinot Saint-George, Primitivo di Gioa, Red Veltliner, Refosco, Rkatsiteli, Royalty, Rubired, Ruby Cabernet, Saint-Emilion, Saint Macaire, Salvador, Sangiovese, Sauvignon blanc, Sauvignon gris, Sauvignon vert, Scarlet, Seibel 5279, Seibel 9110, Seibel 13053, Semillon, Servant, Shiraz, Souzao, Sultana Crimson, Sylvaner, Tannat,

- 32 -

Teroldico, Tinta Madeira, Tinto cao, Touriga, Traminer, Trebbiano Toscano, Troussseau, Valdepenas, Viognier, Walschriesling, White Riesling, and Zinfandel. Rootstock cultivars which can be protected include Couderc 1202, Couderc 1613, Couderc 1616, Couderc 3309, Dog Ridge, Foex 33 EM, Freedom, Ganzin 1 (A x R #1), Harmony, Kober 5BB, LN33, Millardet & de Grasset 41B, Millardet & de Grasset 420A, Millardet & de Grasset 101-14, Oppenheim 4 (SO4), Paulsen 775, Paulsen 1045, Paulsen 1103, Richter 99, Richter 110, Riparia Gloire, Ruggeri 225, Saint-George, Salt Creek, Teleki 5A, *Vitis rupestris* Constantia, *Vitis californica*, and *Vitis girdiana*.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedures. Alternatively, transgenic seeds or propagules (e.g., scion or rootstock cultivars) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart oomycete resistance to plants.

## EXAMPLES

The following examples are provided to illustrate embodiments of the present invention, but they are by no means intended to limit its scope.

### **Example 1 - Construction of Chimeric Gene**

#### **25 Cloning of *gst1* promoter**

The *gst1* promoter region from nucleotides (539 to +48) (Martini et al., "Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional Activation Selectively upon Fungal Infection," Mol. Gen. Genet. 236 (2-3):179-86 (1993), which is hereby incorporated by reference), was PCR amplified using DNA from potato cultivar Atlantic, using a forward primer containing a BamHI site (SEQ. ID. No. 18) as follows:

- 33 -

tgacggatcc taggaagttt cacttttggt gg

32

a reverse primer containing an EcoRI site (SEQ. ID. No. 19) as follows:

5 tagcgaattc tatgtgtggt tggctccct tg

32

and PrimeZyme DNA polymerase (Whatman Biometra, Goettingen, Germany). The DNA was ligated into the LITMUS 38 vector (New England Biolabs, Beverly, MA) and three clones were sequenced on an ABI 377 sequencer at the Cornell BioResource Center. Each clone had two to three nucleotide changes when compared to the published sequence (Martini et al., "Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional Activation Selectively upon Fungal Infection," Mol. Gen. Genet. 236: (2-3) 179-86 (1993), which is hereby incorporated by reference). The changes were most likely due to mistakes made by the polymerase because the promoter is extremely A-T rich and all but one of the changes were in different places in the three clones. One clone, pCPP1308, with a single change in the cis-acting region identified by Martini et al. ("Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional Activation Selectively upon Fungal Infection," Mol. Gen. Genet. 236: (2-3) 179-86 (1993), which is hereby incorporated by reference) was used as the source of the *gstI* promoter in all subsequent constructions.

#### Plant Transformation Constructs

The *gstI:uidA* construct was made by ligating the *gstI* promoter from pCPP1308 into pBI101 (Clontech Labs, Palo Alto, CA). For the *gstI:hrpN* and *gstI:signal sequence:hrpN* constructs (described below), the *gstI* promoter region was engineered to have a 5' HindIII site and a 3' XbaI site by the polymerase chain reaction (PCR) using pCPP1308 as the template. The forward primer had the nucleotide sequence of SEQ. ID. No. 18 and the reverse primer had a nucleotide sequence according to SEQ. ID. No. 20 as follows:

32

- 34 -

For *gstI:hrpN* constructs, the *hrpN* gene of *Erwinia amylovora* (i.e., encoding a hypersensitive response elicitor identified as harpin<sub>Ea</sub>) was engineered to have a 5' XbaI restriction site and a 3' SstI restriction site by PCR using pCPP1084 (Wei et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia Amylovora*," Science 257:85-88 (1992), which is hereby

5 incorporated by reference) as the template. The forward primer had a nucleotide sequence corresponding to SEQ. ID. No. 21 as follows:

ataactctaga accatgggtc tgaatacaag tggg 34

10 and the reverse primer had a nucleotide sequence corresponding to SEQ. ID. No. 22 as follows:

tcatgagctc ttaagccggc ccagcttgcc aagtg 35

15 For *gstI*:signal sequence:*hrpN*, the *hrpN* gene was engineered to have a BamHI site on each end. The forward primer had a nucleotide sequence corresponding to SEQ. ID. No. 23 as follows:

20 tagaggatcc ctgaatacaa gtgggctggg agcg 34

and the reverse primer had a nucleotide sequence corresponding to SEQ. ID. No. 24 as follows:

25 tcatggatcc ttaagccggc ccagcttgcc caagtg 36

The nopaline synthase terminator was extracted from pBI101 by digesting with SstI and EcoRI.

30 The nucleic acid molecule encoding the PR1-b signal sequence (of SEQ. ID. No. 11) was engineered to have XbaI restriction sites on both ends. The forward primer had a nucleotide sequence corresponding to SEQ. ID. No. 25 as follows:



- 35 -

atactctaga ccatgggatt ttttctcttt tca

33

and the reverse primer had a nucleotide sequence corresponding to SEQ. ID. No. 26 as follows:

5

aggtctagag ttttgggcat gagaagagtg

30

The fragment was amplified using pSKG55 as a template (Gopalan et al., "Expression of the *Pseudomonas Syringae* Avirulence Protein AvrB in Plant Cells Alleviates its Dependence on the Hypersensitive Response and Pathogenicity (Hrp) Secretion System in Eliciting Genotype-Specific Hypersensitive Cell Death." Plant Cell 8:1095-1105 (1996), which is hereby incorporated by reference).

PrimeZyme DNA polymerase (Whatman Biometra, Goettingen, Germany) was used with a hot start procedure for amplification of all fragments. The amplified fragments were purified, digested with the appropriate enzymes, and ligated into the binary vector pPZP221 (Hajdukiewicz et al., "The Small Versatile pPZP Family of *Agrobacterium* Binary Vectors for Plant Transformation," Plant Mol. Bio. 25:989-994 (1994), which is hereby incorporated by reference) or intermediate constructs, to build up the final constructs. The proper construction of pCPP1294 (Figure 1) was confirmed by sequencing on an ABI 377 automated sequencer.

The final constructs were transformed into *Agrobacterium tumefaciens* strain GV3101 (Martin et al., "The GUS Reporter System as a Tool to Study Plant Gene Expression," in Gallagher, ed., GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, Academic Press, pp. 23-43 (1992), which is hereby incorporated by reference) by electroporation using a Bio-Rad GenePulser (Bio-Rad Ltd., York, UK).

### **Example 2 - Inoculation with *Peronospora parasitica* Activates *gst1* Transcription in *Arabidopsis***

30

To evaluate the activity of the *gst1* promoter in a plant other than potato, transgenic *Arabidopsis* were constructed containing the *E. coli uidA* gene for  $\beta$ -glucuronidase (GUS) under control of the *gst1* promoter. Histochemical GUS

assays of were performed essentially as described by Martin et al., "The GUS Reporter System as a Tool to Study Plant Gene Expression," in Gallagher, ed., GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, Academic Press, pp. 23-43 (1992), which is hereby incorporated by reference. Uninoculated and inoculated whole small *Arabidopsis* plants were submerged for 30 minutes on ice in six well microtiter plates in a solution of 1.5% freshly prepared paraformaldehyde in 100 mM sodium phosphate buffer, pH 7.2, containing 0.1% Triton X-100. The plants were washed twice for 5 minutes with sodium phosphate buffer pH 7.2. The plants were then submerged in a solution of 2 mM X-gluc (5-bromo-4-chloro-3-indolyl  $\beta$ -D-glucuronide), 50 mM sodium phosphate, pH 7.2, 0.5% Triton X-100. The solution was vacuum infiltrated into the plants and the plants were then incubated for 16 hours in the dark at 37°C. The staining was stopped by rinsing the plants several times in water and the tissue was then cleared by incubating in several changes of 70% ethanol.

Twenty lines were evaluated for GUS expression in uninoculated leaves, leaves inoculated with *Peronospora parasitica* isolate NOCO, and whole plants using a histochemical staining procedure (Martin et al., "The GUS Reporter System as a Tool to Study Plant Gene Expression," in Gallagher, ed., GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, Academic Press, pp 23-43 (1992), which is hereby incorporated by reference). Five lines showed more intense staining of the inoculated areas than the uninoculated areas and two lines showed no visible staining of any plant parts except the inoculated leaves (Figure 2). These results are consistent with those reported for potato and reveal that the *gst1* promoter is pathogen inducible in *Arabidopsis*. No induction of GUS activity was detected in the five lines that responded to *P. parasitica* when inoculated with *Pseudomonas syringae* pv. tomato strain DC3000, even after disease symptoms appeared (results not shown). Previously, it was reported that the *gst1* gene is induced in response to fungi, viruses, and nematodes (Strittmatter et al., "Infections with Various Types of Organisms Stimulate Transcription From a Short Promoter Fragment of the Potato *gst1* Gene," Mol. Plant-Microbe Interact. 9:68-73 (1996), which is hereby incorporated by reference), but results with bacterial pathogens were not reported.

- 37 -

**Example 3 - Pathogen Inducible Expression of *hrpN* in Transgenic *Arabidopsis***

To generate transgenic *Arabidopsis* expressing *hrpN* in a pathogen-inducible manner, plant transformation vectors, pCPP1292 for cytoplasmic localization of HrpN in plants, and pCPP1294 for extracellular localization of HrpN, were constructed. (Figures 3A and 3B). *Arabidopsis* ecotype Columbia (Col-0) was transformed with the two constructs. *Arabidopsis thaliana* ecotype Columbia (Col-0) plants were grown in a growth chamber at 22° C and a 17 hour photoperiod. Plants with primary fluorescence 5-15 cm tall were transformed via a known vacuum infiltration method (protocol available on the Internet at <http://www.bch.msu.edu/pamgreen/vac.htm>, which is hereby incorporated by reference) adapted from Bechtold et al., *C. R. Acad. Sci. Paris* 316:1194-1199 (1993), and Bent et al., *Science* 265:1856-1860 (1994), which are hereby incorporated by reference. Seeds were collected from each plant individually, sterilized and spread on selection plates containing 150 mg/l gentamycin, 0.2 g/l *Arabidopsis* Growth Medium (Lehle Seeds), and 0.7% Phytagar (Gibco BRL, Bethesda, MD). Plates were vernalized for 2 days at 4°C and then moved to a growth chamber maintained at 22° C and 14 hours light. Gentamycin resistant plants were selected after 2 weeks and individual plants were transplanted to soil. Each individual T1 seedling was brought up by single seed descent and individual plant lines were selected for lack of segregation of gentamycin resistance in the T3 generation. Insertion of T-DNA was confirmed by PCR and Southern analysis.

Transgenic *Arabidopsis* lines were inoculated 2 weeks after sowing with a  $5 \times 10^4$  conidiospore suspension of *P. parasitica* isolate NOCO. Flats were covered with a humidity dome and moved to the growth chamber maintained at 18° C, 16 hours light, and 100% humidity. Plants were scored for infection 7 days after inoculation with a disease rating system adapted from Cao et al., "Generation of Broad-Spectrum Disease Resistance by Overexpression of an Essential Regulatory Gene in Systemic Acquired Resistance," *Proc. Natl. Acad. Sci. USA* 95:6531-6536 (1998), which is hereby incorporated by reference. A rating of 1, 0 conidiophores present; 2, 0-5 conidiophores present; 3, 6-20 conidiophores on a few leaves; 4, 6-20 conidiophores on all leaves; 5, 20 or more conidiophores present on all leaves. Inoculated leaves were stained with lactophenol-trypan blue (Keogh et al.,

“Comparison of Histological and Physiological Responses to *Phakopsora pachyrhizi* in Resistant and Susceptible Soybean,” Trans. Br. Mycol. Soc. 74:329-333 (1980), which is hereby incorporated by reference) to observe the extent of fungal colonization under the microscope.

5               Plants were selected that lacked segregation of antibiotic resistance in the T3 generation. Lines containing the *gst1:hrpN* construct (“GN lines”) lines were tested for resistance to *P. parasitica* isolate NOCO in an initial screen.

                  Thirty lines containing the *gst1*:signal sequence:*hrpN* construct (“GSSN lines”) were tested for resistance to *P. parasitica* isolate NOCO in an initial  
10   screen. All but one of the lines was free of any signs of the oomycete ten days after inoculation. Ten GSSN lines were chosen for further study and inoculated by spraying with a conidiospore suspension ( $5 \times 10^4$  spores/ml) of *P. parasitica* NOCO. Northern analysis revealed that expression of *hrpN* was induced by *P. parasitica* 2  
15   days after inoculation with strong induction at 4 days (Figure 3A). A range of expression levels were observed among the ten lines, line GSSN 8-4 was chosen for further study as it displayed the highest level of expression. Production of the harpin<sub>Ea</sub> protein in inoculated plants was confirmed by immuno-blot analysis.

                  RNA was isolated from inoculated plants over a 4 day interval to analyze *hrpN* gene expression. RNA was isolated from 1g of plant tissue as  
20   described by Carpenter et al., “Preparation of RNA, in *Arabidopsis* Protocols,” (Martinez-Zapater, JM. and Salinas, J., eds.), Humana Press, Totowata, New Jersey, pp. 85-89 (1998). Twenty micro-gram samples were separated by formaldehyde-agarose gel electrophoresis and blotted onto Hybond N+ membranes (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK). Hybridizations and  
25   washing were performed according to Church et al., “Genomic Sequencing,” Proc. Natl. Acad. Sci. USA 81:1991-1995 (1984), which is hereby incorporated by reference, using P<sup>32</sup> labeled *hrpN* DNA as a probe.

                  The *Arabidopsis* lines GSSN 8-4 (test), Col-0 WT (wild type, control), and Col-0 EV (empty vector, control) were inoculated by drop inoculation with a  
30   conidiospore suspension ( $5 \times 10^4$  spores/ml) of *P. parasitica*. Plants were maintained in a growth chamber (16 hours of light, 18° C, 100% humidity) and were scored for infection ten days post inoculation. Nearly all (29 out of 30) 8-4 plants were free of

any signs of *P. parasitica* (Figure 4A). Trypan blue staining showed that growth of the oomycete was strongly inhibited in GSSN 8-4 plants. Extensive hyphal growth was evident in Col-0 WT and Col-0 EV plants (Figure 4B).

Plants were rated for disease severity based on the number of  
5 conidiophores per leaf. Nearly all GSSN 8-4 plants received a disease rating of 1 with only one being scored 3. The majority of the Col-0 WT and Col-0 EV plants were rated 5, the remainder were rated 4 (Figure 5).

This example demonstrates that pathogen inducible expression of the harpin<sub>Ea</sub> hypersensitive response elicitor of *Erwinia amylovora* in transgenic plants is  
10 a potentially useful strategy for engineering plants for disease resistance. Challenge with *Peronospora parasitica* resulted in accumulation of *hrpN* mRNA, production of harpin<sub>Ea</sub> protein, and resistance to *P. parasitica*. Upon challenge by *P. parasitica*, it is believed that the transgenic plants most likely mount a hypersensitive response at the site of inoculation, conferring resistance. Subsequently the plants may develop  
15 systemic resistance.

For the purposes of the present invention, the *gst1* promoter was most applicable to the *Arabidopsis/P. parasitica* pathosystem since it is well documented that transcription from *gst1* is activated by other oomycete pathogens (Martini et al., "Promoter Sequences of a Potato Pathogenesis-related Gene Mediate Transcriptional  
20 Activation Selectively upon Fungal Infection," Mol. Gen. Genet. 236: (2-3) 179-86 (1993), which is hereby incorporated by reference). Additionally, it has been reported that *gst1* activation is stimulated by ascomycete, viral, and nematode infection and mycorrhization (Strittmatter et al., "Infections with Various Types of Organisms Stimulate Transcription From a Short Promoter Fragment of the Potato *gst1* Gene,"  
25 Mol. Plant-Microbe Interact. 9:68-73 (1996), which is hereby incorporated by reference). Therefore, it is possible that both *gst1:hrpN* and *gst1:signal* sequence:*hrpN* constructs may also confer resistance against ascomycete, virus, and nematode infection, as well as mycorrhization.

Although the invention has been described in detail for the purpose of  
30 illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

All of the references designated as being incorporated herein by reference are intended to be incorporated in their entirety unless specific portions thereof have been identified with particularity.

**WHAT IS CLAIMED:**

1. A chimeric gene comprising:  
a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide,  
a promoter operably linked 5' to the first DNA molecule to induce transcription of the first DNA molecule in response to activation of the promoter by an oomycete, and  
a 3' regulatory region operably linked to the first DNA molecule.
2. The chimeric gene according to claim 1 further comprising:  
a second DNA molecule encoding a secretion signal polypeptide, the second DNA molecule being operably linked between the promoter and the first DNA molecule.
3. The chimeric gene according to claim 2, wherein the second DNA molecule encodes a secretion signal polypeptide comprising an amino acid sequence of SEQ. ID. No. 11, SEQ. ID. No. 13, SEQ. ID. No. 15, or SEQ. ID. No. 17.
4. The chimeric gene according to claim 3, wherein the second DNA molecule comprises a nucleotide sequence of nt 8-110 from SEQ. ID. No. 10, SEQ. ID. No. 12, SEQ. ID. No. 14, or SEQ. ID. No. 16.
5. The chimeric gene according to claim 1, wherein the promoter is a *gstI* promoter.
6. The chimeric gene according to claim 1, wherein the *gstI* promoter comprises a nucleotide sequence of SEQ. ID. No. 9 or effective fragments thereof.
7. The chimeric gene according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

8. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

9. The chimeric gene according to claim 8, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 3.

10. The chimeric gene according to claim 9, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 4.

11. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

12. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

13. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

14. The chimeric gene according to claim 13, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 1.

15. The chimeric gene according to claim 14, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 2.

16. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.



17. The chimeric gene according to claim 16, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 5.
18. The chimeric gene according to claim 17, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 6.
19. The chimeric gene according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.
20. The chimeric gene according to claim 19, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 7.
21. The chimeric gene according to claim 20, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 8.
22. An expression system comprising a vector into which is inserted a chimeric gene according to claim 1.
23. A host cell comprising a chimeric gene according to claim 1.
24. The host cell according to claim 23, wherein the host cell is a bacterial cell or a plant cell.
25. The host cell according to claim 24, wherein the bacterial cell is an *Agrobacterium* cell.
26. The host cell according to claim 24, wherein the host cell is a plant cell.
27. The host cell according to claim 26, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

28. The host cell according to claim 26, wherein the chimeric gene further comprises

a second DNA molecule encoding a secretion signal polypeptide, the second DNA molecule being operably linked between the promoter and the first DNA molecule.

29. The host cell according to claim 26, wherein the promoter is a *gstI* promoter.

30. A transgenic plant resistant to disease resulting from oomycete infection, the transgenic plant comprising:

a chimeric gene according to claim 1, wherein the promoter induces transcription of the first DNA molecule in response to infection of the plant by an oomycete.

31. The transgenic plant according to claim 30, wherein the chimeric gene further comprises

a second DNA molecule encoding a secretion signal, the second DNA molecule being operably linked between the promoter and the first DNA molecule.

32. The transgenic plant according to claim 31, wherein the second DNA molecule encodes a secretion signal polypeptide comprising an amino acid sequence of SEQ. ID. No. 11, SEQ. ID. No. 13, SEQ. ID. No. 15, or SEQ. ID. No. 17.

33. The transgenic plant according to claim 32, wherein the second DNA molecule comprises a nucleotide sequence of nt 8-110 from SEQ. ID. No. 10, SEQ. ID. No. 12, SEQ. ID. No. 14, or SEQ. ID. No. 16.

34. The transgenic plant according to claim 30, wherein the *gstI* promoter comprises a nucleotide sequence of SEQ. ID. No. 9 or effective fragments thereof.

35. The transgenic plant according to claim 30, wherein the oomycete is a species of *Plasmopara*, *Phytophthora*, *Peronospora*, *Pseudoperonospora*, *Bremia*, *Sclerospora*, *Aphanomyces*, *Pythium*, or *Albugo*.

36. The transgenic plant according to claim 30, wherein the transgenic plant is selected from a group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

37. The transgenic plant according to claim 36, wherein the transgenic plant is a grape plant.

~~38. The transgenic plant according to claim 37, wherein the oomycete is selected from the group consisting of *Plasmopara viticola* and *Phytophthora parasitica*.~~

39. The transgenic plant according to claim 36, wherein the transgenic plant is a tobacco plant.

40. The transgenic plant according to claim 39, wherein the oomycete is selected from the group consisting of *Peronospora tabacina*, *Pythium* spp., and *Phytophthora* spp.

41. The transgenic plant according to claim 30, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

42. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

43. The transgenic plant according to claim 42, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 3.

44. The transgenic plant according to claim 44, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 4.

45. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

46. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

47. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

48. The transgenic plant according to claim 47, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 1.

49. The transgenic plant according to claim 48, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 2.

50. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

51. The transgenic plant according to claim 50, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 5.

52. The transgenic plant according to claim 51, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 6.

53. The transgenic plant according to claim 41, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

54. The transgenic plant according to claim 53, wherein the first DNA molecule encodes a hypersensitive response elicitor protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 7.
55. The transgenic plant according to claim 54, wherein the first DNA molecule comprises a nucleotide sequence of SEQ. ID. No. 8.
56. The transgenic plant according to claim 30, wherein the chimeric gene is stably inserted into the genome of the transgenic plant.
57. A method of making a recombinant plant cell comprising:  
transforming a plant cell with a chimeric gene according to claim 1 under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter.
58. A method of making a plant resistant to disease resulting from oomycete infection, the method comprising:  
transforming a plant cell with a chimeric gene according to claim 1 under conditions effective to yield transcription of the first DNA molecule in response to oomycete-induced activation of the promoter and  
regenerating a plant from the transformed plant cell.
59. The method according to claim 58, wherein said transforming is performed under conditions effective to insert the chimeric gene into the genome of the plant cell.
60. The method according to claim 58, wherein said transforming is *Agrobacterium* mediated.
61. The method according to claim 58, wherein said transforming comprises:  
propelling particles at the plant cell under conditions effective for the particles to penetrate into the cell interior and  
introducing an expression vector comprising the chimeric gene into the plant cell interior.

62. The method according to claim 58, wherein the chimeric gene further comprises  
a second DNA molecule encoding a secretion signal, the second DNA molecule being operably linked between the promoter and the first DNA molecule.
63. The method according to claim 58, wherein the promoter is a *gst1* promoter.
64. The method according to claim 58, wherein the oomycete is a species of *Plasmopara*, *Phytophthora*, *Peronospora*, *Pseudoperonospora*, *Bremia*, *Sclerospora*, *Aphanomyces*, *Pythium*, or *Albugo*.
65. The method according to claim 58, wherein the transgenic plant is selected from the group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.
66. The method according to claim 65, wherein the transgenic plant is a grape plant.
67. The method according to claim 66, wherein the oomycete is selected from the group consisting of *Plasmopara viticola* and *Phytophthora parasitica*.
68. The method according to claim 65, wherein the transgenic plant is a tobacco plant.
69. The method according to claim 68, wherein the oomycete is selected from the group consisting of *Peronospora tabacina*, *Phytophthora* spp., and *Pythium* spp.

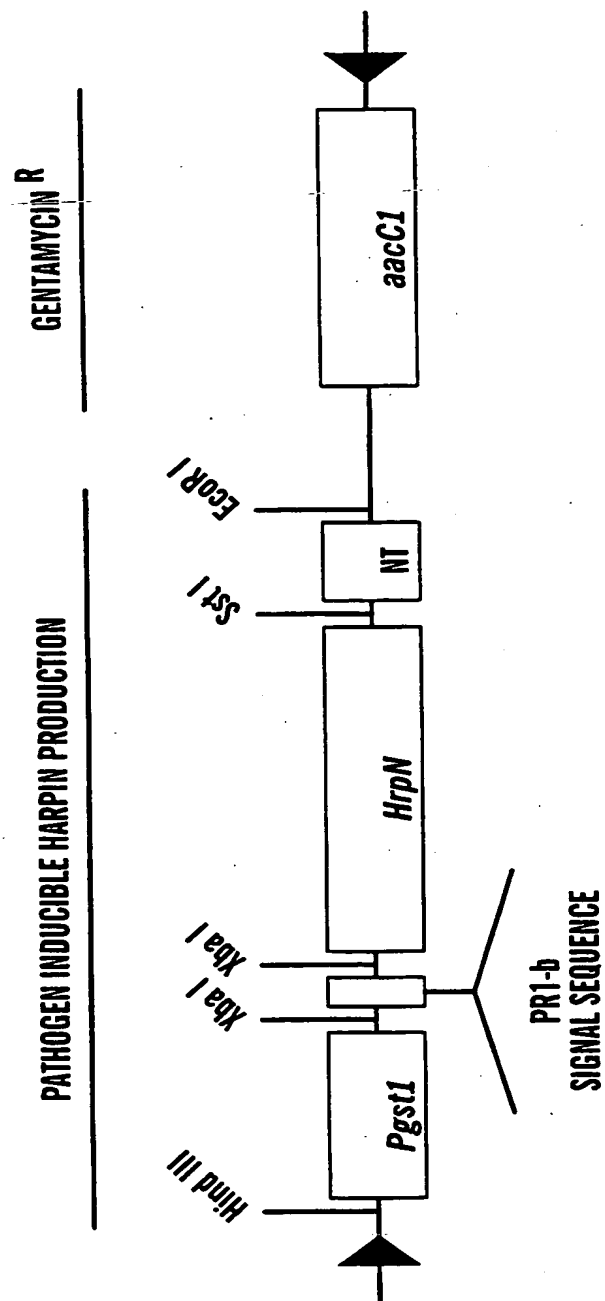
70. The method according to claim 58, wherein the hypersensitive response elicitor protein or polypeptide derives from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

71. A transgenic plant seed obtained from the transgenic plant according to claim 30.

72. A transgenic plant scion or rootstock cultivar obtained from the transgenic plant according to claim 30.

**THIS PAGE BLANK (USPTO)**

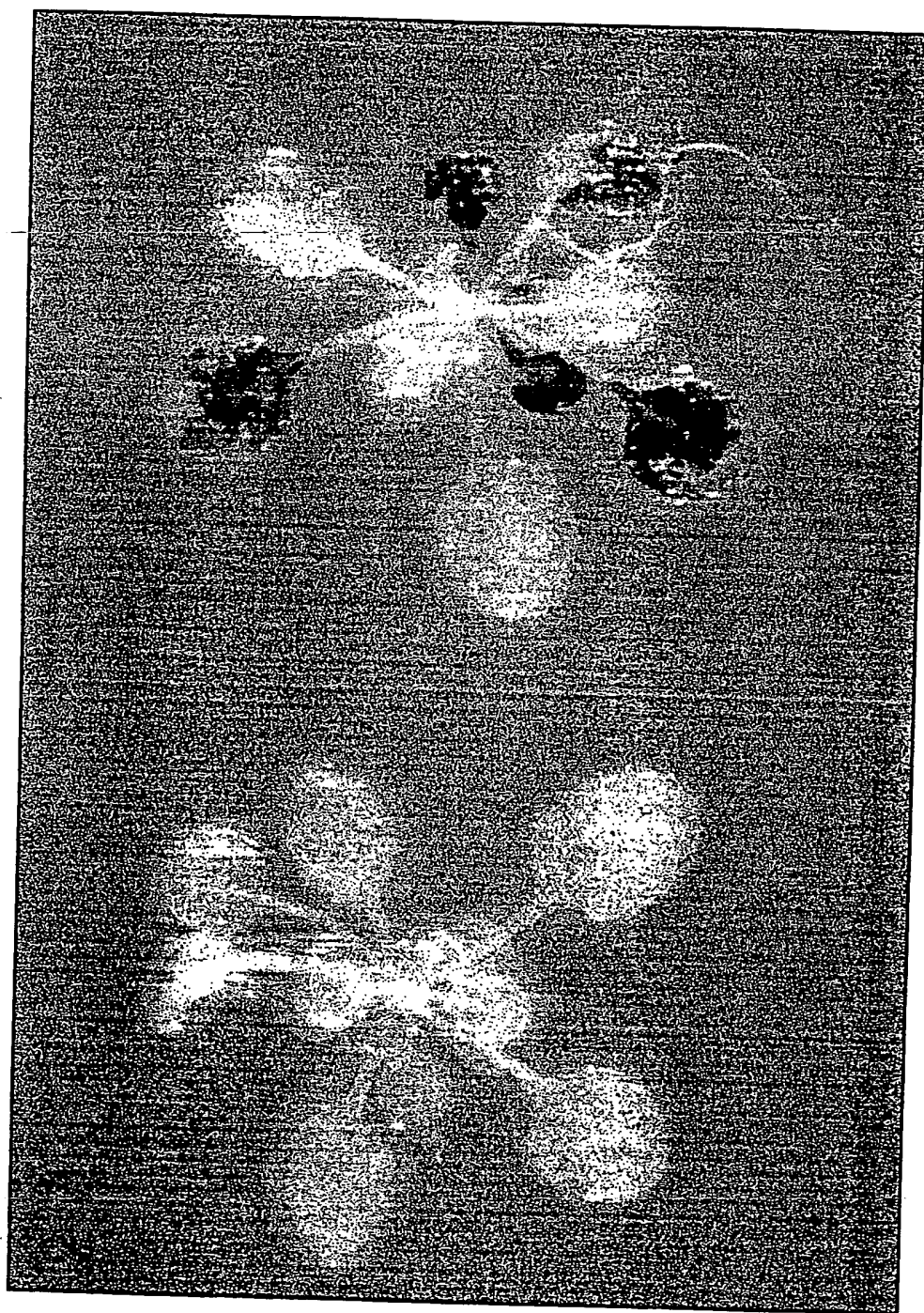




**FIG. 1**

**THIS PAGE BLANK (USPTO)**

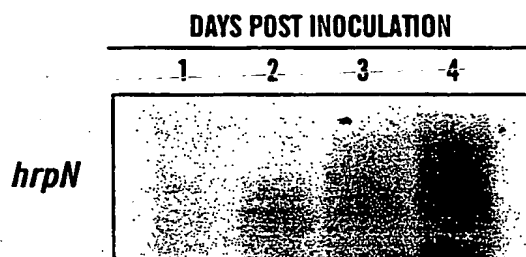
2/6



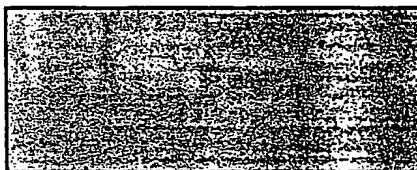
**FIG. 2**

**THIS PAGE BLANK (USPTO)**

3/6



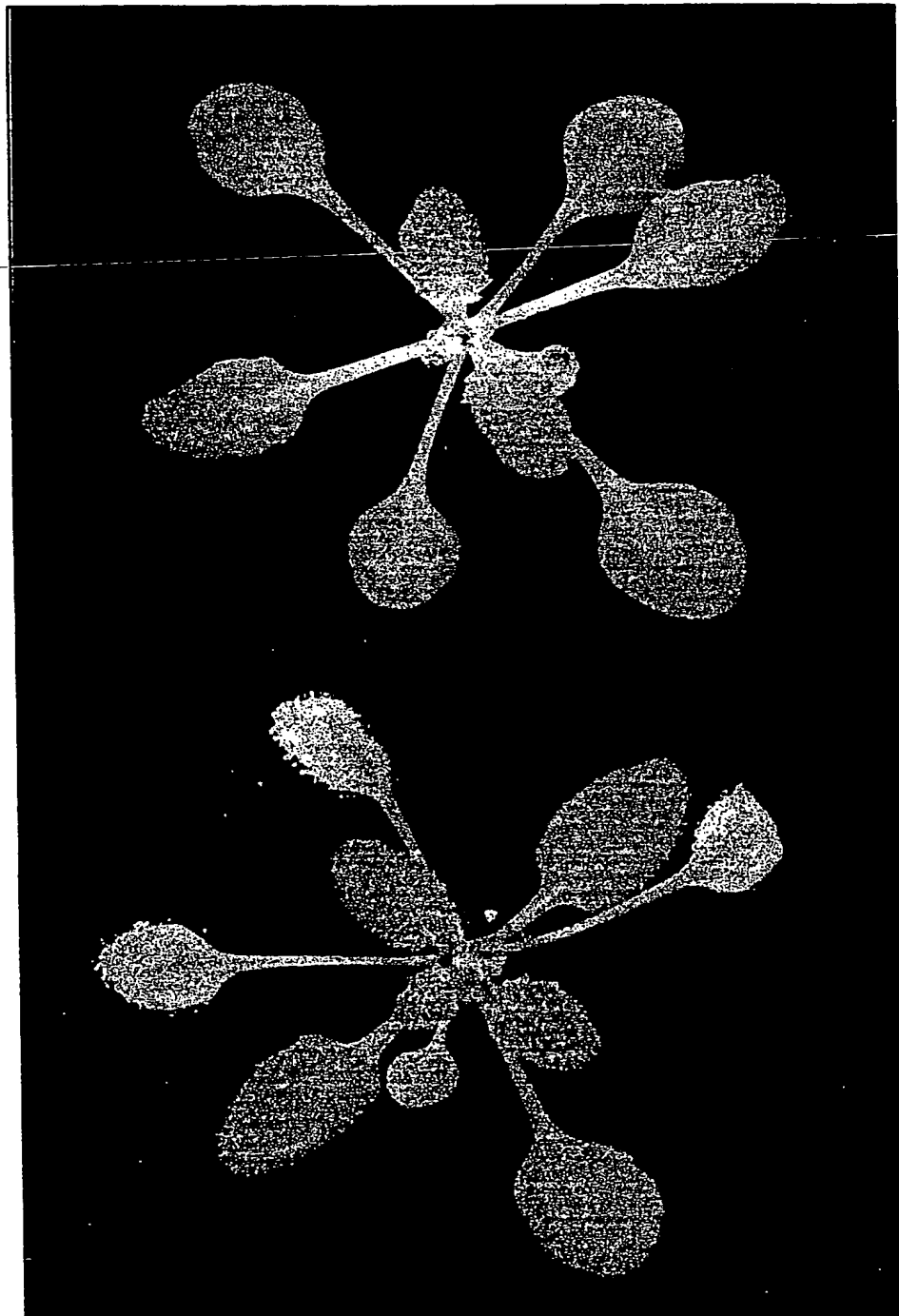
**FIG. 3A**



**FIG. 3B**

**THIS PAGE BLANK (USPTO)**

4/6

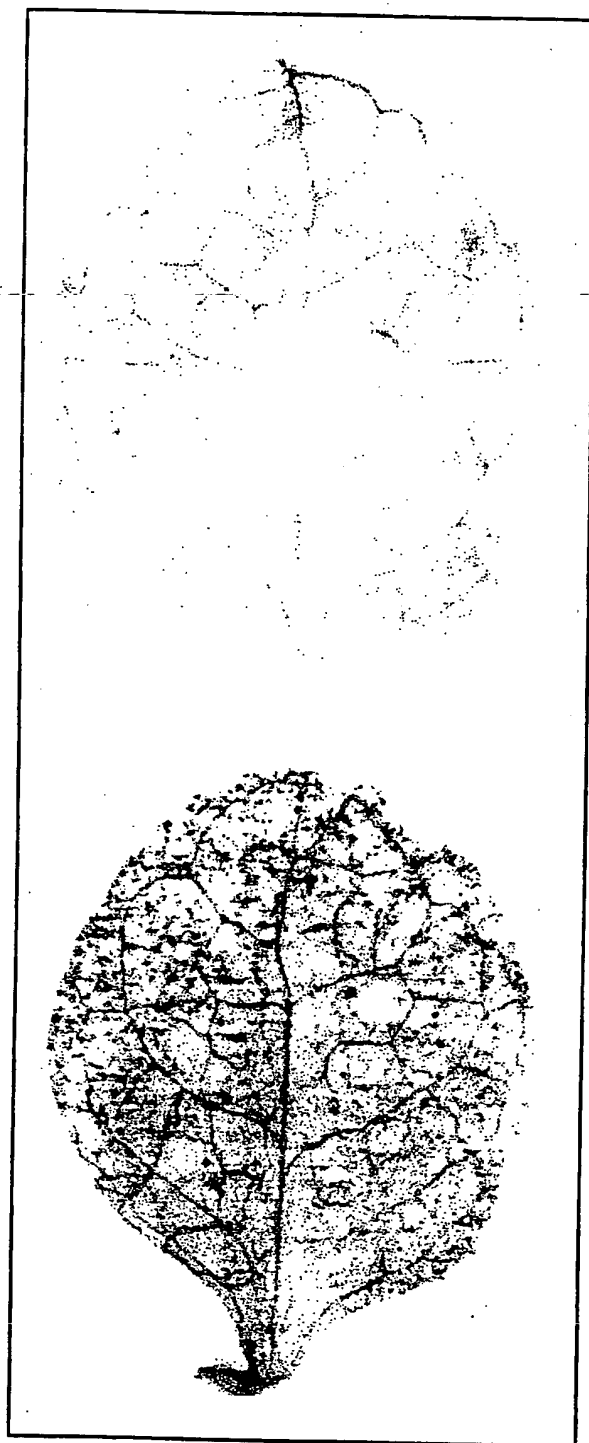


**FIG. 4A**

**THIS PAGE BLANK (USPTO)**



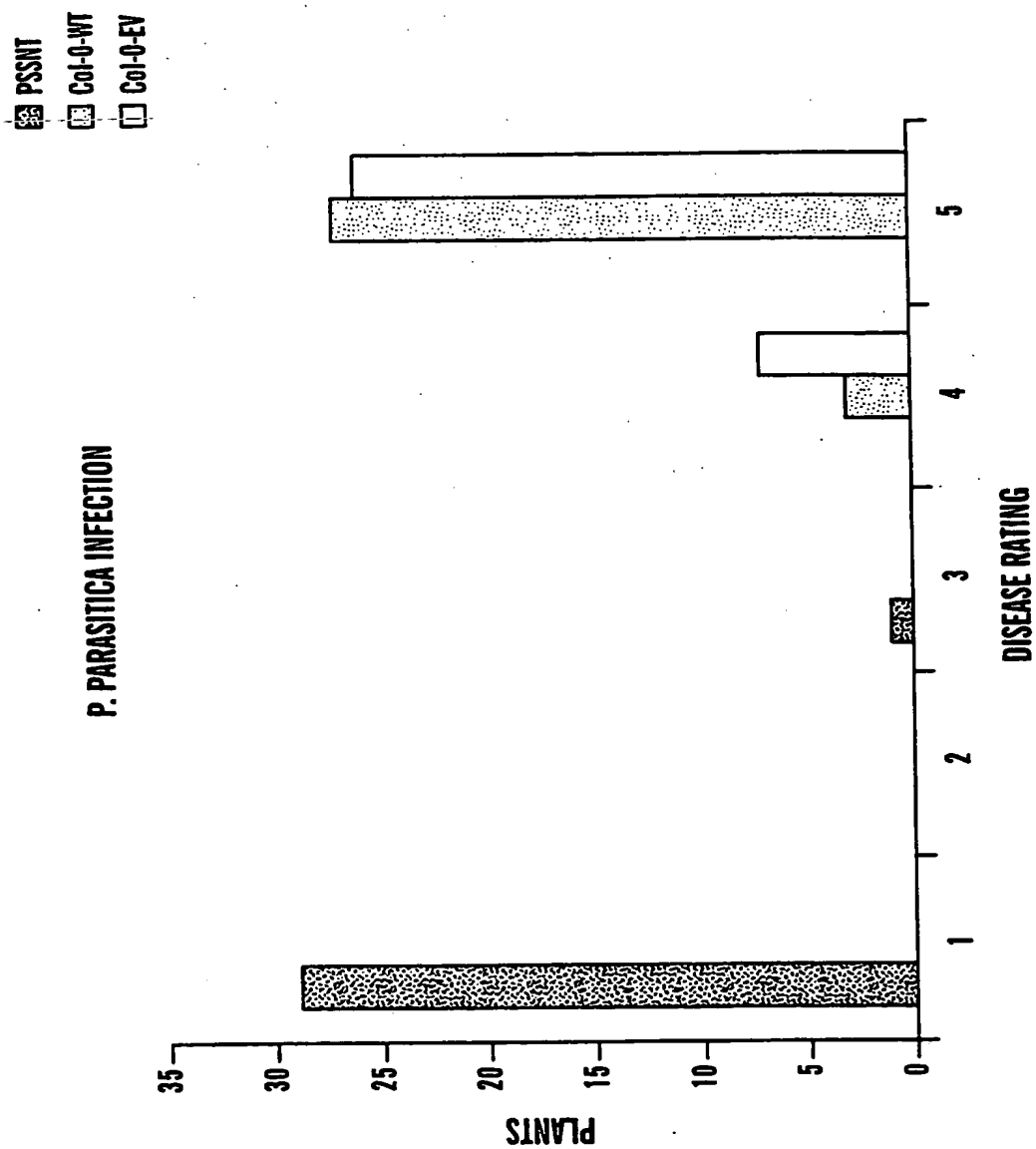
5/6



**FIG. 4B**

**THIS PAGE BLANK (USPTO)**

6/6



**FIG. 5**

THIS PAGE BLANK (USPTO)

## SEQUENCE LISTING

&lt;110&gt; Cornell Research Foundation, Inc.

<120> OOMYCETE-RESISTANT TRANSGENIC PLANTS BY VIRTUE OF  
PATHOGEN-INDUCED EXPRESSION OF A HETEROLOGOUS  
HYPERSENSITIVE RESPONSE ELICITOR

&lt;130&gt; 19603/2502

&lt;140&gt;

&lt;141&gt;

&lt;150&gt; 60/178,565

&lt;151&gt; 2000-01-26

&lt;160&gt; 26

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 338

&lt;212&gt; PRT

<213> *Erwinia chrysanthemi*

&lt;400&gt; 1

Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser
1				5					10					15	

Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser
		20					25						30		

Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr
		35					40					45			

Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu
	50					55					60				

Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser
65					70					75					80

Phe	Gly	Asn	Gly	Ala	Gln	Gly	Ala	Ser	Asn	Leu	Leu	Ser	Val	Pro	Lys
			85						90					95	

Ser	Gly	Gly	Asp	Ala	Leu	Ser	Lys	Met	Phe	Asp	Lys	Ala	Leu	Asp	Asp
			100					105					110		

Leu	Leu	Gly	His	Asp	Thr	Val	Thr	Lys	Leu	Thr	Asn	Gln	Ser	Asn	Gln
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

**THIS PAGE BLANK (USPTO)**

115	120	125
Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met		
130	135	140
Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly		
145	150	155
Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly		
	165	170
Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu		
	180	185
Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala		
195	200	205
Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val		
210	215	220
Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp		
225	230	235
Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp		
	245	250
Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys		
	260	265
Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln		
275	280	285
Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr		
290	295	300
Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala		
305	310	315
Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala		
	325	330
Asn Ala		

&lt;210&gt; 2

&lt;211&gt; 2141

&lt;212&gt; DNA

**THIS PAGE BLANK (USPTO)**



<213> *Erwinia chrysanthemi*

&lt;400&gt; 2

```

cgattttacc cgggtgaacg tgctatgacc gacagcatca cgggtattcga caccggttacg 60
gcgttttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgcccga atccggcgctc 120
gatctgggtat ttcagtttgg ggacaccggg cgtgaactca tgatgcagat tcagccgggg 180
cagcaatata ccggcatggt gcgcacgctg ctgcgctcgtc gttatcagca ggccggcagag 240
tgcatgggct gccatctgtg cctgaacggc agcgaatgtat tgatcctctg gtggccgctg 300
ccgtcggatc ccggcagtta tccgcagggtg atcgaacgtt tgtttgaact ggccgggaatg 360
acgttgccgt cgtatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc 420
cgatcattaa gataaaggcg gcttttttta ttgcaaacg gtaacgggtga ggaaccgttt 480
caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgctg 540
ggcatccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca 600
aattacgatc aaagcgcaca tcggcggtga tttgggcgtc tccgggtctgg ggctgggtgc 660
tcagggactg aaaggactga attccgcggc ttcacgctg gggtccagcg tggataaact 720
gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct 780
ggcgccagggg ctgggcgccg gctcgaaggg gctggggatg agcaatcaac tgggccagtc 840
tttcggcaat ggcgcgcagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcgccga 900
tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac 960
caagctgact aaccagagca accaactggc taattcaatg ctgaacgccg gccagatgac 1020
ccagggtaat atgaatgctg tcggcagcgg tgtgaacaac gcactgtcgt ccattctcgg 1080
caacgggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggtctt 1140
gcagggcctg agcggcgcgg gtgcattcaa ccagttgggt aatgccatcg gcatggcgct 1200
ggggcagaat gctgcgctga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260
ccgccacttt gtagataaag aagatcgcgg catggcgaaa gagatcggcc agtttatgga 1320
tcagtatccg gaaatatctg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380
gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg 1440
cgccagcatg gacaaattcc gtcaggcgat gggatgatc aaaagcgcgg tggcggtga 1500
taccggcaat accaacctga acctgcgtgg cgcggcggtg gcatcgctgg gtatcgatgc 1560
ggctgtcgtc ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620
atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680
ttattatgcg gtttatgctg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740
acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttcctc 1800
gtcgtcaga ttgcggcggt gatggggaac gccgggtgga atatagagaa actcgccggc 1860
cagatggaga cacgtctgct ataaatctgt gccgtaacgt gtttctatcc gccccttag 1920
cagatagatt gcggtttctg aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980
gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040
aaaatagggc agtttttgct tggatatcgt ggggtgttcc ggcctgacaa tcttgagttg 2100
gttcgtcatc atctttctcc atctgggcga cctgatcggt t

```

2141

&lt;210&gt; 3

&lt;211&gt; 403

&lt;212&gt; PRT

<213> *Erwinia amylovora*

&lt;400&gt; 3

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser

**THIS PAGE BLANK (USPTO)**

1                      5                      10                      15  
 Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
                     20                      25                      30  
 Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
                     35                      40                      45  
 Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
                     50                      55                      60  
 Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
                     65                      70                      75                      80  
 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
                     85                      90                      95  
 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
                     100                      105                      110  
 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
                     115                      120                      125  
 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
                     130                      135                      140  
 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
                     145                      150                      155                      160  
 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
                     165                      170                      175  
 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu  
                     180                      185                      190  
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly  
                     195                      200                      205  
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly  
                     210                      215                      220  
 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu  
                     225                      230                      235                      240  
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln  
                     245                      250                      255  
 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln

**THIS PAGE BLANK (USPTO)**

<210> 4  
<211> 1288  
<212> DNA  
<213> *Erwinia amylovora*

<400> 4						
aagcttcggc	atggcacggt	tgaccgttgg	gtcggcaggg	tacgtttgaa	ttattcataa	60
gaggaatacg	ttatgagtct	gaatacaagt	gggctgggag	cgtcaacgat	gcaaatttct	120
atcggcgggtg	cgggcggaaa	taacgggttg	ctgggtacca	gtcgcagaa	tgctgggttg	180
ggtggcaatt	ctgcactggg	gctgggcggc	ggtaatcaaa	atgataccgt	caatcagctg	240
gctggcttac	tcaccggcat	gatgatgatg	atgagcatga	tgggcggtgg	tgggctgatg	300
ggcggtggtc	taggcggtgg	cttaggtaat	ggcttgggtg	gctcaggtgg	cctgggcgaa	360
ggactgtcga	acgcgctgaa	cgatatgtta	ggcggttcgc	tgaacacgct	gggctcgaaa	420
ggcggcaaca	ataccacttc	aacaacaaat	tccccgctgg	accaggcgct	gggtattaac	480
tcaacgtccc	aaaacgacga	ttccacctcc	ggcacagatt	ccacctcaga	ctccagcgac	540
ccgatgcagc	agctgctgaa	gatgttcagc	gagataatgc	aaagcctgtt	tgggtgatggg	600
caagatggca	cccagggcag	ttcctctggg	ggcaagcagc	cgaccgaagg	cgagcagaac	660
gcctataaaa	aaggagtcac	tgatgcgctg	tcgggcctga	tgggtaatgg	tctgagccag	720

**THIS PAGE BLANK (USPTO)**

ctccttggca acgggggact gggaggtggt cagggcggtgta atgctggcac ggggtcttgac 780  
 gggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccggtgga ctaccagcag 840  
 ttaggtaacg ccgtgggtac cggtatcggg atgaaagcgg gcattcaggc gctgaatgat 900  
 atcgggtacgc acaggcacag ttcaaccctg tctttcgtca ataaaggcga tcgggcgatg 960  
 gcgaaggaaa tcggtcagtt catggaccag tctcctgagg tgtttgga ggcgcagtac 1020  
 cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc 1080  
 aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc 1140  
 atgatcaaaa ggcccatggc ggggtgatacc ggcaacggca acctgcaggc acgcggtgcc 1200  
 ggtgggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca 1260  
 cttggcaagc tgggcgcggc ttaagctt 1288

&lt;210&gt; 5

&lt;211&gt; 341

&lt;212&gt; PRT

<213> Pseudomonas syringae

&lt;400&gt; 5

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
 1 5 10 15  
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser  
 20 25 30  
 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met  
 35 40 45  
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala  
 50 55 60  
 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val  
 65 70 75 80  
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
 85 90 95  
 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met  
 100 105 110  
 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
 115 120 125  
 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
 130 135 140  
 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
 145 150 155 160

**THIS PAGE BLANK (USPTO)**



Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
 165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Il  
 180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
 195 200 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
 210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser  
 225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp  
 245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val  
 260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
 275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
 290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
 305 310 315 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
 325 330 335

Asn Gln Ala Ala Ala  
 340

<210> 6

<211> 1026

<212> DNA

<213> *Pseudomonas syringae*

<400> 6

atgcagagtc tcagtcttaa cagcagctcg ctgcaaacc cggcaatggc ccttgctctg 60  
 gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagttgtc 120  
 gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga 180  
 aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc 240  
 atcgctgcgc tggacaagct gatccatgaa aagctcggtg acaacttcgg cgcgtctgcg 300

**THIS PAGE BLANK (USPTO)**

```

gacagcgccct cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc 360
aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac 420
gatatgccga tgctgaacaa gatcgcgag ttcattggatg acaatcccgc acagtttccc 480
aagccggact cgggctcctg ggtgaacgaa ctcaaggaag acaacttcct tgatggcgac 540
gaaacggctg cgttcggttc ggcactcgac atcattggcc agcaactggg taatcagcag 600
agtgcgctg gcagtcctggc agggacgggt ggaggtcttg gcactccgag cagtttttcc 660
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccgggtcc cggtgacagc 720
ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780
tcgggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgagac cggtagctcg 840
gcgaatggcg gacagtcgc tcaggatcct gatcagttgc tgggaggctt gctgctcaag 900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960
gcgcaaactc ccaccttgct ggtcagtacg ctgctgcaag gcaccgcaa tcaggctgca 1020
gcctga 1026

```

&lt;210&gt; 7

&lt;211&gt; 344

&lt;212&gt; PRT

<213> *Pseudomonas solanacearum*

&lt;400&gt; 7

```

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1           5           10           15

```

```

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
20           25           30

```

```

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
35           40           45

```

```

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
50           55           60

```

```

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65           70           75           80

```

```

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
85           90           95

```

```

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
100          105          110

```

```

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
115          120          125

```

```

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
130          135          140

```

**THIS PAGE BLANK (USPTO)**

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
 145 150 155 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
 165 170 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
 180 185 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
 195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
 210 215 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
 245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
 260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
 275 280 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
 290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
 305 310 315 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
 325 330 335

Gln Ser Thr Ser Thr Gln Pro Met  
 340

<210> 8

<211> 1035

<212> DNA

<213> *Pseudomonas solanacearum*

<400> 8

atgtcagtcg gaaacatcca gagcccgctg aacctccccg gtctgcagaa cctgaacctc 60  
 aacaccaaca ccaacagcca gcaatcgggc cagtcctgtc aagacctgat caagcaggtc 120

**THIS PAGE BLANK (USPTO)**

```

gagaaggaca tcctcaacat catcgagcc ctcgtgcaga aggccgcaca gtcggcgggc 180
ggcaacaccg gtaacaccgg caacgcgccc gcgaaggacg gcaatgccaa cgcggggcgcc 240
aacgaccgga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc 300
ggcaacgctg acgacgccaa caaccaggat ccgatgcaag cgctgatgca gctgctggaa 360
gacctggtga agctgctgaa ggcgggccctg cacatgcagc agcccggcgg caatgacaag 420
ggcaacggcg tgggcggtgc caacggcgcc aagggtgccg ggggccaggg cggcctggcc 480
gaagcgctgc aggagatcga gcagatcctc gccagctcg gcgggcgcgg tgctggcgcc 540
ggcgggcgcg gtggcggtgt cggcggtgct ggtggcgcgg atggcggtc cggtgcggtg 600
ggcgcgaggc gtgcgaacgg cgcgcagggc ggcaatggcg tgaacggcaa ccaggcgaac 660
ggccccgaga acgcaggcga tgtcaacggt gccaacggcg cggatgacgg cagcgaagac 720
caggggcgcc tcaccggcgt gctgcaaaaag ctgatgaaga tcctgaacgc gctggtgcag 780
atgatgcagc aaggcggcct cggcgggcgcc aaccaggcgc agggcggtc gaagggtgcc 840
ggcaacgcct cgcgggcttc cggcgcgaa cggggcgcg accagcccgg ttcggcggtg 900
gatcaatcgt cgggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc 960
gtccagatcc tgcagcagat gctggcgggc cagaacggcg gcagccagca gtccacctcg 1020
acgcagccga tgttaa
1035

```

&lt;210&gt; 9

&lt;211&gt; 696

&lt;212&gt; DNA

<213> *Solanum tuberosum*

&lt;400&gt; 9

```

gaattcagga agaattttgt aggttcaact aaattatata tatatatata aaaaaataaa 60
aattattaga cgcttcgact atttacttac tttaaaattt gaattttcgt acgaataaaa 120
ttatttgta gagaaaagtc ttttagctat tcacatgcta ggaagtttca cttttggtgg 180
atcagtgatt gtatattatt taatatatat caattttctc atcaaaactga aaatgaaaga 240
taaaattaat attaaaaact ccattcattt taatttattg tcatgttttg acttgatcca 300
aaatctaaca atttaaaagg ttttaaaattt ttgtgctttt ttttaatta aaaaatgtgc 360
aaatatatta aaatatattt tttaaatttt atactaaaaa acatgtcaca tgaatatttg 420
aaattataaa attatcaaaa ataaaaaaag aatattttct taacaaatta aaattgaaaa 480
tatgataaat aaattaaact attctatcat tgatttttct agccaccaga tttgaccaa 540
cagtgggtga catgagcaca taagtcattt ttattgtatt ttattactca ctccaaaaat 600
ataggggaata tgtttactac ttaatttagt caaatataat tttatattag aataattgaa 660
tagtcaaaca agaaacttta atgcattcctt attttt
696

```

&lt;210&gt; 10

&lt;211&gt; 110

&lt;212&gt; DNA

<213> *Nicotiana tabacum*

&lt;400&gt; 10

```

tctagaccat gggatttttt ctcttttcac aaatgccctc attttttctt gtgtcgacac 60
ttctcttatt cctaataata tctcactctt ctcatgcccc aaactctaga
110

```

**THIS PAGE BLANK (USPTO)**



<210> 11  
 <211> 34  
 <212> PRT  
 <213> Nicotiana tabacum

<400> 11  
 Met Gly Phe Phe Leu Phe Ser Gln Met Pro Ser Phe Phe Leu Val Ser  
           1                  5                  10                  15  
 Thr Leu Leu Leu Phe Leu Ile Ile Ser His Ser Ser His Ala Gln Asn  
                   20                  25                  30

Ser Arg

<210> 12  
 <211> 102  
 <212> DNA  
 <213> Nicotiana tabacum

<400> 12  
 atgggatttt ttctcttttc acaaatgcc tcattttttc ttgtctctac acttctctta 60  
 ttcttaataa tatctcactc ttctcatgcc caaaactctc aa 102

<210> 13  
 <211> 34  
 <212> PRT  
 <213> Nicotiana tabacum

<400> 13  
 Met Gly Phe Phe Leu Phe Ser Gln Met Pro Ser Phe Phe Leu Val Ser  
           1                  5                  10                  15  
 Thr Leu Leu Leu Phe Leu Ile Ile Ser His Ser Ser His Ala Gln Asn  
                   20                  25                  30

Ser Gln

<210> 14  
 <211> 90  
 <212> DNA  
 <213> Nicotiana tabacum

<400> 14

**THIS PAGE BLANK (USPTO)**

atgggatttg ttctcttttc acaattgcct tcattttcttc ttgtctctac acttctctta 60  
 ttcttagtaa tatcccactc ttgccgtgcc 90

<210> 15

<211> 30

<212> PRT

<213> Nicotiana tabacum

<400> 15

Met Gly Phe Val Leu Phe Ser Gln Leu Pro Ser Phe Leu Leu Val Ser  
 1 5 10 15

Thr Leu Leu Leu Phe Leu Val Ile Ser His Ser Cys Arg Ala  
 20 25 30

<210> 16

<211> 75

<212> DNA

<213> Nicotiana tabacum

<400> 16

atggagagag ttaataatta taagttgtgc gtggcattgt tgatcatcag catggtgatg 60  
 gcaatggcgg cggca 75

<210> 17

<211> 25

<212> PRT

<213> Nicotiana tabacum

<400> 17

Met Glu Arg Val Asn Asn Tyr Lys Leu Cys Val Ala Leu Leu Ile Ile  
 1 5 10 15

Ser Met Val Met Ala Met Ala Ala Ala  
 20 25

<210> 18

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

**THIS PAGE BLANK (USPTO)**

&lt;400&gt; 18

tgacggatcc taggaagttt cacttttggt gg

32

&lt;210&gt; 19

&lt;211&gt; 32

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: primer

&lt;400&gt; 19

tagcgaattc tatgtgtggt tggctctccct tg

32

&lt;210&gt; 20

&lt;211&gt; 32

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: primer

&lt;400&gt; 20

tacgtctaga tatgtgtggt tggctctccct tg

32

&lt;210&gt; 21

&lt;211&gt; 34

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: primer

&lt;400&gt; 21

atactctaga accatgggtc tgaatacaag tggg

34

&lt;210&gt; 22

&lt;211&gt; 35

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: primer

**THIS PAGE BLANK (USPTO)**

<400> 22  
tcatgagctc ttaagccggc ccagcttgcc aagtg

35

<210> 23  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 23  
tagaggatcc ctgaatacaa gtgggctggg agcg

34

<210> 24  
<211> 36  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 24  
tcatggatcc ttaagccgcg cccagcttgc caagtg

36

<210> 25  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 25  
atactctaga ccatgggatt ttttctcttt tca

33

<210> 26  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

**THIS PAGE BLANK (USPTO)**



WO 01/55347

PCT/US01/02579

<400> 26

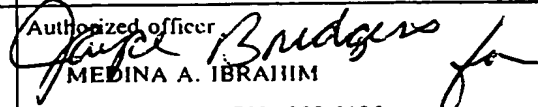
aggtctagag ttttgggcat gagaagagtg

30

**THIS PAGE BLANK (USPTO)**

# INTERNATIONAL SEARCH REPORT

PCT/US01/02579

<b>CLASSIFICATION OF SUBJECT MATTER</b> INT. CL. (1979) E01N 5/04; 15/09, 15/29, 15/31, 15/82; A01H 5/00 US CL. (Please See Extra Sheet) According to International Patent Classification (IPC) or to both national classification and IPC				
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/69.1, 320.1, 419, 468; 536/23.1, 23.6, 23.7, 24.1; 800/278, 279, 287, 288, 298, 295 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN CAS, WEST2.0 search terms: hypersensitive, elicitor, oomycete, gst1 promoter, transgenic plants, disease resistance, fungal pathogen				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
Y	US 5,859,332 A (STRITTMATTER et al) 12 January 1999 (12.01.99), see entire document, especially columns 3-5, 11-18, 23-26.	1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72		
A	HART et al. Regulated Inactivation of Homologous Gene Expression in Transgenic Nicotiana sylvestris Plants Containing a Defense-related Tobacco Chitinase Gen. Mol. Gene. Genet. 1992, Vol. 235, No 2-3, pages 179-186, see entire document.	1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72		
Y	WEI et al. Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia amylovora. Science 03 July 1992, Vol. 257, pages 85-88, see entire document.	1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72		
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.				
<table border="0"> <tr> <td>           * Special categories of cited documents:            *A* document defining the general state of the art which is not considered to be of particular relevance            *E* earlier document published on or after the international filing date            *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)            *O* document referring to an oral disclosure, use, exhibition or other means            *P* document published prior to the international filing date but later than the priority date claimed         </td> <td>           *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention            *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone            *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art            *Z* document member of the same patent family         </td> </tr> </table>			* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *Z* document member of the same patent family
* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *Z* document member of the same patent family			
Date of the actual completion of the international search 14 MAY 2001		Date of mailing of the international search report 14 JUN 2001		
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer  MEDINA A. IBRAHIM Telephone No. (703) 308-0196		

# INTERNATIONAL SEARCH REPORT

PCT/US01/02579

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/69.1, 320.1, 419, 468; 536/23.1, 23.6, 23.7, 24.1; 800/278, 279, 287, 288, 298, 295

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1, 5-6, 22-26, 29-30, 34-40, 56-61, 63-69, 71-72, drawn to a chimeric gene comprising a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a specified *gst1* promoter, and a method of making a transgenic plant which is resistant to disease from oomycete by expressing said chimeric gene.

Group II, claim(s) 1-4, 22-26, 28, 31-33, 62, drawn to a chimeric gene comprising a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a second DNA molecule encoding specific sequences of secretion signal polypeptides, a *gst1* promoter, and a method of making a transgenic plant which is resistant to disease from oomycete by expressing said chimeric gene.

Group III, claim(s) 1, 7-21, 22-26, 27, 41-55, 70, drawn to a chimeric gene comprising a first DNA molecule encoding a hypersensitive response elicitor protein or polypeptide of a specified sequence, a *gst1* promoter, and a method of making a transgenic plant which is resistant to disease from oomycete by expressing said chimeric gene.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
27 September 2001 (27.09.2001)

PCT

(10) International Publication Number  
**WO 01/70988 A2**

(51) International Patent Classification<sup>7</sup>: **C12N 15/29**,  
15/82, 15/11, 1/21, 5/10, A01H 5/00, G01N 33/68, A01N  
65/00, 63/00

(74) Agents: **GOLDMAN, Michael, L.** et al.; Nixon Peabody  
LLP, Clinton Square, P.O. Box 31051, Rochester, NY  
14603-1051 (US).

(21) International Application Number: **PCT/US01/08728**

(22) International Filing Date: 19 March 2001 (19.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/191,649 23 March 2000 (23.03.2000) US  
60/250,710 1 December 2000 (01.12.2000) US

(71) Applicant: **EDEN BIOSCIENCE CORPORATION**  
[US/US]; 11816 North Creek Parkway N., Bothell, WA  
98011-8205 (US).

(72) Inventors: **SONG, Xiaoling**; 14110 NE 179th Street, Apt.  
75, Woodinville, WA 98072 (US). **FAN, Hao**; 19712 6th  
Drive S.E., Bothell, WA 98012 (US). **WEI, Zhong-Min**;  
8230 125th Court, Kirkland, WA 98034 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,  
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— without international search report and to be republished  
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: **RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS AND USES THEREOF**

(57) Abstract: The present invention is directed to an isolated protein which serves as a receptor in plants for a plant pathogen hypersensitive response elicitor. Also disclosed are nucleic acid molecules encoding such receptors as well as expression vectors, host cells, transgenic plants, and transgenic plant seeds containing such nucleic acid molecules. Both the protein and nucleic acid can be used to identify agents targeting plant cells to enhance a plant's receptivity to treatment with a hypersensitive response elicitor and to directly impart plant growth enhancement as well as resistance against disease, insects, and stress.

**WO 01/70988 A2**

**THIS PAGE BLANK (USPTO)**

## RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS AND USES THEREOF

This application claims benefit of U.S. Provisional Patent Application  
5 Serial Nos. 60/191,649, filed March 23, 2000 and 60/250,710, filed December 1,  
2000.

### FIELD OF THE INVENTION

10 The present invention relates to receptors for hypersensitive response  
elicitors and uses thereof.

### BACKGROUND OF THE INVENTION

15 Plants have evolved a complex array of biochemical pathways that  
enable them to recognize and respond to environmental signals, including pathogen  
infection. There are two major types of interactions between a pathogen and plant --  
compatible and incompatible. When a pathogen and a plant are compatible, disease  
generally occurs. If a pathogen and a plant are incompatible, the plant is usually  
20 resistant to that particular pathogen. In an incompatible interaction, a plant will  
restrict pathogen proliferation by causing localized necrosis, or death of tissues, to a  
small zone surrounding the site of infection. This reaction by the plant is defined as  
the hypersensitive response ("HR") (Kiraly, Z. "Defenses Triggered by the Invader:  
Hypersensitivity," Plant Disease: An Advanced Treatise 5:201-224 J. G. Horsfall and  
25 E. B. Cowling, eds. Academic Press, New York (1980); (Klement "Hypersensitivity,"  
Phytopathogenic Prokaryotes 2:149-177, M.S. Mount and G. H. Lacy, eds. Academic  
Press, New York (1982)). The localized cell death not only contains the infecting  
pathogen from spreading further but also leads to a systemic resistance preventing  
subsequent infections by other pathogens. Therefore, HR is a common form of plant  
30 resistance to diseases caused by bacteria, fungi, nematodes, and viruses.

A set of genes designated as *hrp* (Hypersensitive Response and  
Pathogenicity) is responsible for the elicitation of the HR by pathogenic bacteria,  
including *Erwinia* spp, *Pseudomonas* spp, *Xanthomonas* spp, and *Ralstonia*  
*solanacearum* (Willis et al. "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-

- Microbe Interact. 4:132-138 (1991), Bonas, U. "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology, Vol. 192, Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms. J. L. Dangl, ed. Springer-Verlag, Berlin (1994); Alfano et al., "Bacterial Pathogens in Plants: Life Up Against the Wall," Plant Cell 8:1683-98 (1996).
- Typically, there are multiple *hrp* genes clustered in a 30-40 kb DNA. Mutation in any one of the *hrp* genes will result in the loss of bacterial pathogenicity in host plants and the HR in non-host plants. On the basis of genetic and biochemical characterization, the function of the *hrp* genes can be classified into three groups: 1) structural genes encoding extracellularly located HR elicitors, for example harpin of *Erwinia amylovora* (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992)); 2) secretion genes encoding a secretory apparatus for exporting HR elicitors and other proteins from the bacterial cytoplasm to the cell surface or extracellular space (Van Gijsegem et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993); He et al. "Pseudomonas syringae pv. Syringae harpin<sub>pss</sub>: A Protein that is Secreted Via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255 (1993); Wei et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7985-67 (1993), Arlat et al. "PopA1, a Protein which Induces a Hypersensitive-Like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994), Galan et al., "Cross-talk between Bacterial Pathogens and their Host Cells," Ann. Rev. Cell Dev. Biol. 12:221-55 (1996); Bogdanove et al., "*Erwinia amylovora* Secretes Harpin via a Type-III Pathway and Contains a Homolog of yopN of Yersinia," J. Bacteriol. 178:1720-30 (1996); Bogdanove et al., "Homology and Functional Similarity of a *hrp*-linked Pathogenicity Operon, *dspEF*, of *Erwinia amylovora* and the *avrE* locus of *Pseudomonas syringae* pathovar tomato," Proc Natl Acad Sci USA 95:1325-30 (1998)); and 3) regulatory genes that control the expression of *hrp* genes (Wei, Z. M., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992); Wei et al., "*hrpL* Activates *Erwinia amylovora hrp* Genes in Response to



- 3 -

Environmental Stimuli," J. Bacteriol. 174:1875-82 (1995); Xiao et al., "A Single Promoter Sequence Recognized by a Newly Identified Alternate Sigma Factor Directs Expression of Pathogenicity and Host Range Determinants in *Pseudomonas syringae*," J. Bacteriol. 176:3089-91 (1994); Kim et al., "The hrpA and hrpC Operons of *Erwinia amylovora* Encode Components of a Type III Pathway that Secretes Harpin," J. Bacteriol. 179:1690-97 (1997); Kim et al., "HrpW of *Erwinia amylovora*, a New Harpin that Contains a Domain Homologous to Pectate Lyases of a Distinct Class," J. Bacteriol. 180:5203-10 (1998); Wengelnik et al., "HrpG, A Key hrp Regulatory Protein of *Xanthomonas campestris* pv. *Vesicatoria* is Homologous to Two Component Response Regulators," Mol. Plant-Microbe Interact. 9:704-12 (1996)). Because of their role in interactions between plants and microbes, *hrp* genes have been a focus for bacterial pathogenicity and plant defense studies.

In addition to the local defense response, HR also activates the defense system in uninfected parts of the same plant. This results in a general systemic resistance to a secondary infection termed Systemic Acquired Resistance ("SAR") (Ross, R. F. "Systemic Acquired Resistance Induced by Localized Virus Infections in Plants," Virology 14:340-58 (1961); Malamy et al., "Salicylic Acid and Plant Disease Resistance," Plant J. 2:643-654 (1990)). SAR confers long-lasting systemic disease resistance against a broad spectrum of pathogens and is associated with the expression of a certain set of genes (Ward et al. "Coordinate Gene Activity in Response to Agents that Induce Systemic Acquired Resistance," Plant Cell 3:1085-94 (1991)). SAR is an important component of the disease resistance of plants and has long been of interest, because the potential of inducing the plant to protect itself could significantly reduce or eliminate the need for chemical pesticides. SAR can be induced by biotic (microbes) and abiotic (chemical) agents (Gorlach et al. "Benzothiadiazole, a Novel Class of Inducers of Systemic Acquired Resistance, Activates Gene Expression and Disease Resistance in Wheat," Plant Cell 8:629-43 (1996)). Historically, weak virulent pathogens were used as a biotic inducing agent for SAR. Non-virulent plant growth promotion bacteria (PGPR) were also reported to be able to induce resistance of some plants against various diseases. Biotic agent-induced SAR has been the subject of much research, especially in the late 70s and early 80s. Only very limited success was achieved, however, due to: 1) inconsistency

of the performance of living organisms in different environmental conditions;  
2) considerable concerns regarding the unpredictable consequences of the intentional  
introduction of weakly virulent pathogens into the environment; and 3) the technical  
complication of applying a living microorganism into a variety of environmental  
5 conditions. To overcome the limitations of using living organisms to induce SAR,  
scientists have long been looking for an HR elicitor derived from a pathogen for SAR  
induction. With the advancement of molecular biology, the first proteinaceous HR  
elicitor with broad host spectrum was isolated in 1992 from *Erwinia amylovora*, a  
pathogenic bacterium causing fire blight in apple and pear. The HR elicitor was  
10 named "harpin". It consists of 403 amino acids with a molecular weight about  
40 kDa. The harpin protein is heat-stable and glycine-rich with no cysteine. The gene  
encoding the harpin protein is contained in a 1.3 kB DNA fragment located in the  
middle of the *hrp* gene cluster. Harpin is secreted into the extracellular space and is  
very sensitive to proteinase digestion. Since the first harpin was isolated from  
15 *Erwinia amylovora*, several harpin or harpin-like proteins have been isolated from  
other major groups of plant pathogenic bacteria. In addition to the harpin of *Erwinia*  
*amylovora*, the following harpin or harpin-like proteins have been isolated and  
characterized: HrpN of *Erwinia chrysanthemi*, *Erwinia carotovora* (Wei et al.  
"Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen  
20 *Erwinia amylovora*," Science, 257:85 (1992)), and *Erwinia stewartii*; HrpZ of  
*Pseudomonas syringae* (He et al, "Pseudomonas syringae pv. *Syringae* harpin<sub>pss</sub>: A  
Protein that is Secreted Via the Hrp Pathway and Elicits the Hypersensitive Response  
in Plants," Cell 73:1255 (1993)), PopA of *Ralstonia solanacearum*, (Arlat et al.  
"PopA1, a Protein which Induces a Hypersensitive-Like Response on Specific Petunia  
25 Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO  
J. 13:543-53 (1994)); HrpW of *Erwinia amylovora* (Kim et al., "HrpW of *Erwinia*  
*amylovora*, a New Harpin that Contains a Domain Homologous to Pectate Lyases of a  
Distinct Class," J. Bacteriol. 180:5203-10 (1998)), and *Pseudomonas syringae*. All of  
the currently described harpin or harpin-like proteins share common characteristics.  
30 They are heat-stable and glycine-rich proteins with no cysteine amino acid residue,  
are very sensitive to digestion by proteinases, and elicit the HR and induce resistance  
in many plants against many diseases. Based on their shared biochemical and

biophysical characteristics as well as biological functions, these HR elicitors from different pathogenic bacteria belong to a new protein family – i.e. the harpin protein family. The described characteristics, especially their ability to induce HR in a broad range of plants, distinguish the harpin protein family from other host specific  
5 proteinaceous HR elicitors, for example elicitors from *Phytophthora* spp (Bonnet et al., “Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants,” Eur. J. Plant Path. 102:181-92 (1996); Keller, et al. “Physiological and Molecular Characteristics of Elicitin-Induced Systemic Acquired Resistance in Tobacco,” Plant Physiol 110:365-76 (1996)) or avirulence proteins (such as Avr9) from *Cladosporium*  
10 *fulvum*, which are only able to elicit the HR in a specific variety or species of a plant.

In nature, when certain bacterial infections occur, harpin protein is expressed and then secreted by the bacteria, signaling the plant to mount a defense against the infection. Harpin serves as a signal to activate plant defense and other physiological systems, which include SAR, growth enhancement, and resistance to  
15 certain insect damage.

The current understanding of critical plant molecules that may have a significant role in interacting with elicitors and then triggering a sequential signal transduction cascade is described as follows.

## 20 **Interaction of Plant Resistance Genes (R) and Pathogen Avirulence Genes (avr)**

The concept of gene-for-gene interaction is that “for each gene determining resistance (R gene) in the host, there is a corresponding gene determining avirulence in the pathogen (avr gene)”. In this model, pathogen avirulence genes  
25 generate a specific ligand molecule, called an elicitor. Only plants carrying the matching resistance gene respond to this elicitor and invoke the HR. In the past few years, several disease-resistance, R genes, have been cloned and sequenced. It was expected that R genes might encode components involved in signal recognition or signal transduction pathways that ultimately lead to defense responses. The cloned R  
30 genes could be grouped into four classes: (1) cytoplasmic protein kinase; (2) protein kinases with an extracellular domain; (3) cytoplasmic proteins with a region of leucine-rich repeats and a nucleotide-binding site; and (4) proteins with a region of

leucine-rich repeats that appear to encode extracellular proteins. (Review in Bent, A.F. "Plant Disease Resistance Genes: Function Meets Structure," Plant Cell 8:1757-71 (1996); Baker B., et al., "Signaling in Plant-Microbe Interactions," Science 276:726-33 (1997)). The first R gene cloned, Pto, encodes a serine/threonine protein kinase. The protein product of Pto directly interacts with the cognate avirulence gene protein, AvrPro, which has been demonstrated in a yeast two-hybrid system. It was shown that only co-existence of both AvrPro and Pto proteins could elicit HR in plants (Tang et al., "Initiation of Plant Disease Resistance by Physical Interaction of AvrPto and Pto kinase," Science 274:2060-63 (1996); Scofield et al., "Molecular Basis of Gene-for-Gene Specificity in Bacterial Speck Disease of Tomato," Science 274:2063-65 (1996); Zhou et al., "The Pto kinase Conferring Resistance to Tomato Bacterial Speck Disease Interacts with Proteins that Bind a cis-element of Pathogenesis-related Genes," EMBO J. 16:3207-18 (1997)). The results from cloned R genes support the view that plant-pathogen interactions involve protein-protein interactions. Syringolide, a water-soluble, low-molecular-weight elicitor, triggers a defense response in soybean cultivars carrying the Rpg4 disease-resistance gene. A 34-KDa protein has been isolated from soybean and is considered to be the physiological active syringolide receptor (Ji et al., "Characterization of a 34-kDa Soybean Binding Protein for the syringolide Elicitors," Proc. Natl. Acad. Sci. USA 95:3306-11 (1998)).

#### **Putative Binding Factor of Elicitin**

Elicitins are a family of small proteins secreted by *Phytophthora* species that have a high degree of homology. Pure elicitors alone can cause a hypersensitive response, a local cell death, and trigger systemic acquired resistance in tobacco and other plants (Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path. 102:181-92 (1996); Keller, et al. "Physiological and Molecular Characteristics of Elicitin-Induced Systemic Acquired Resistance in Tobacco," Plant Physiol 110:365-76 (1996)). However, the spectrum of HR elicitation and induced systemic resistance in plants is much narrower than that achieved by harpin family elicitors. Like harpin, elicitors induce a series of metabolic events in tobacco cells, including the accumulation of phytoalexins, ethylene

- 7 -

production, transmembrane electrolyte leakage, H<sub>2</sub>O<sub>2</sub> accumulation, and expression of plant defense related genes (Yu L, et al., "Elicitins from *Phytophthora* and Basic Resistance in Tobacco," Proc. Natl. (1995); Keller et al., "Pathogen-Induced Elicitin Production in Transgenic Tobacco Generates a Hypersensitive Response and

5 Nonspecific Disease Resistance," The Plant Cell 11:223-35 (1999)). A putative receptor-like binding factor has been identified in tobacco plasma membrane, which has a specific high-affinity to the cryptogin, one member of the elicitin family (Wendehenne, et al., "Evidence for Specific, High-Affinity Binding Sites for a Proteinaceous Elicitor in Tobacco Plasma Membrane," FEBS Letters 374:203-207

10 (1995)). Recently, it was found that 2 basic elicittins (i.e. cryptogin and cinnamomin) and two acidic elicittins (i.e. capsicein and parasiticein) were able to interact with the same binding sites on tobacco plasma membranes (Bourque et al., "Comparison of Binding Properties and Early Biological Effects of Elicitins in Tobacco Cells," Plant Physiol. 118:1317-26 (1998)). However, the gene of the receptor-like factor has not

15 been isolated.

#### Putative Binding Factor of Glycoprotein Elicitors

A 42 kDa glycoprotein elicitor has been isolated from *Phytophthora*

20 *megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f. sp. *glycinea* Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991)). An oligopeptide of 13 amino acids within the glycoprotein ("Pep-13") was able to induce a response in plants like that achieved by the full glycoprotein. A high affinity-binding pattern has

25 been observed in parsley microsomal membranes with an isotope labeled oligopeptide. There are estimated to be about 1600 to 2900 binding sites per cell with evidence indicating that a low abundant protein receptor of the Pep-13 is localized in the plasma membrane (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense

30 Responses," Cell 78:449-60 (1994)).

### Harpin Protein Binding Factors

Harpin proteins, which elicit HR in a variety of different nonhost plants, have been isolated from plant pathogens (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992)). A family of harpin proteins has been identified from plant bacterial pathogens. All of them have similar biological activities. It is well documented that harpin protein can induce plants to produce active oxygen, change ion flux, lead to local cell death, and induce systemic acquired resistance ("SAR") (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992); He et al., "*Pseudomonas syringae* pv. *syringae* Harpin<sub>pss</sub>: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-66 (1993); Baker, C.J., et al., "Harpin, an Elicitor of the Hypersensitive Response in Tobacco Caused by *Erwinia amylovora*, Elicits Active Oxygen Production in Suspension Cells," Plant Physiol. 102:1341-44 (1993)). No harpin protein binding factor has been isolated so far. It was reported that an amphipathic protein, named HRAP, isolated from sweet pepper could dissociate harpin<sub>pss</sub> in multimeric form (*hrpZ* from *Pseudomonas syringae*). The biological activity of the HRAP is believed to be its ability to intensify harpin<sub>pss</sub>-mediated hypersensitive response. HRAP protein does not bind to harpin<sub>pss</sub> directly (Chen et al., "An Amphipathic Protein from Sweet Pepper can Dissociate Harpin<sub>pss</sub> Multimeric Forms and Intensify the Harpin<sub>pss</sub> -Mediated Hypersensitive Response," Physiological & Molecular Pathology 52:139-49 (1998)). Using a fluorochrome tagged antibody to harpin to examine the interaction of harpin<sub>pss</sub> and tobacco suspension cells, it was found that harpin<sub>pss</sub> interacted with the cultured cells, but not with protoplasts with the cell walls being digested and removed. It was interpreted that harpin<sub>pss</sub> was localized in the outer portion of the plant cell, probably on the cell wall. However, it was not ruled out that the binding factor was located on the plasma membrane.

The present invention seeks to identify receptors for hypersensitive response elicitor proteins or polypeptides and uses of such receptors.

- 9 -

## SUMMARY OF THE INVENTION

The present invention is directed to an isolated protein which serves as a receptor in plants for a plant pathogen hypersensitive response elicitor. Also  
5 disclosed are nucleic acid molecules encoding such receptors as well as expression vectors, host cells, transgenic plants, and transgenic plant seeds containing such nucleic acid molecules.

The protein of the present invention can be used with a method of identifying agents targeting plant cells by forming a reaction mixture including the  
10 protein and a candidate agent, evaluating the reaction mixture for binding between the protein and the candidate agent, and identifying candidate compounds which bind to the protein in the reaction mixture as plant cell targeting agents.

The nucleic acid molecule of the present invention can be used in a method of identifying agents targeting plant cells by forming a reaction mixture  
15 including a cell transformed with the nucleic acid molecule of the present invention and a candidate agent, evaluating the reaction mixture for binding between protein produced by the host cell and candidate agent, and identifying candidate compounds which bind to the protein or the host cell in the reaction mixture as plant cell targeting agents.

20 Another aspect of the present invention relates to a method of enhancing a plant's receptivity to treatment with hypersensitive response elicitors by providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule of the present invention.

The present invention is also directed to a method of imparting disease  
25 resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants by providing a transgenic plant or transgenic plant seed transformed with a DNA construct effective to silence expression of a nucleic acid molecule encoding a receptor in accordance with the present invention.

The discovery of the present invention has great significance. This  
30 putative receptor protein can be used as a novel way to screen for new inducers of plant resistance against insect, disease, and stress, and of growth enhancement. This protein is the first step toward the understanding of the harpin induced signal transduction pathway in plants. Further studies of this pathway will provide more

possible targets for new plant vaccine and growth enhancement products development. In addition, this protein can serve as an anchor providing a new way to target anything to the plant cells.

## BRIEF DESCRIPTION OF THE DRAWINGS

5

Figure 1 shows a yeast two-hybrid screening with the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin) and a schematic representation of the interaction between harpin and a cDNA encoded polypeptide. Harpin is fused to LexA protein which contains a DNA binding domain ("BD"). The cDNA encoded polypeptide is fused to the GAL4 transcription activation domain ("AD"). This interaction targets the activation domain to two different LexA-dependent promoters with consequent activation of the transcription of the *HIS3* and *lacZ* reporter genes.

10

Figures 2A-B show that the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin) is a good yeast two-hybrid bait. Reporter genes were not expressed in yeast strain L40 containing plasmids expressing the LexA - harpin fusion in combination with plasmids expressing the GAL4 activation domain alone, or fused to unrelated protein. Therefore, harpin is not autoactive in this yeast two-hybrid system. In addition, reporter genes were not expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-harpin fusion in combination with plasmids expressing LexA alone, or fused to unrelated protein. Figure 2A shows a  $\beta$ -galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 2B shows a synthetic minimal ("SD") media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

20

25

Figures 3A-B show the interaction between HrBP1 (hypersensitive response elicitor binding protein 1) and a hypersensitive response elicitor (i.e. harpin) is specific. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-HrBP1 fusion in combination with plasmids expressing LexA fused to hypersensitive response elicitor (i.e. harpin), but were not expressed in combination with LexA alone, or LexA fused to unrelated proteins.

30



- 11 -

Figure 3A is a  $\beta$ -galactosidase assay where the blue color indicates the expression of *lacZ* reporter gene. Figure 3B is an SD media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

5                    Figures 4A-B show the interaction of HrBP1 and a hypersensitive response elicitor (i.e. harpin) in another orientation. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the LexA - HrBP1 fusion in combination with plasmids expressing GAL4 activation domain fused to harpin, but were not expressed in combination with GAL4 activation domain alone, or GAL4  
10                    activation domain fused to unrelated proteins. Therefore, interaction between harpin and HrBP1 is specific. Figure 4A shows a  $\beta$ -galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 4B shows an SD media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

15                    Figure 5 shows the gene structure of HrBP1 and a schematic representation of the exons and introns of the HrBP1 gene. When comparing the HrBP1 cDNA sequence with the *Arabidopsis thaliana* genomic DNA sequence published in a public database, four exons and three introns were discovered.

                    Figure 6 shows a Northern blot using RNA probe complementary to  
20                    bases 651-855 of HrBP1 coding region (SEQ. ID. No. 9).

                    Figures 7A-B show that the interaction between rHrBP1 (R6) and harpin is specific. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-rHrBP1 fusion in combination with plasmids expressing LexA fused to harpin or harpin 137-180 amino acids, but were  
25                    not expressed in combination with LexA alone, LexA fused to unrelated proteins, or fused to harpin 210-403 amino acids. Figure 7A shows a  $\beta$ -galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 7B shows a SD media plate, which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

30                    Figure 8 shows the constructs used to "knockout" HrBP1 gene in *Arabidopsis*.

Figure 11A-B show the height of wild type and HrBP1 overexpressing tobacco plants 52 days after they were transferred to soil. Figure 11A is a picture taken 52 days after plants were transferred to soil. Figure 11B shows average height of 8 plants per line.

Figure 12A-B show a TMV assay results on wild type and HrBP1 overexpressing tobacco plants. Figure 12A is a picture taken 3 days after TMV inoculation. Figure 12B shows the average virus lesion diameter from 5 plants per line 3 days after TMV inoculation.

20 The present invention is directed to isolated receptors for hypersensitive response elicitor proteins or polypeptides. Also disclosed are DNA molecules encoding such receptors as well as expression systems, host cells, and plants containing such molecules. Uses of the receptors themselves and the DNA molecules encoding them are disclosed. The receptor for a hypersensitive response  
25 elicitor from a plant pathogen can be from a monocot or a dicot.

One example of such a receptor is that found in *Arabidopsis thaliana* which has the amino acid sequence of SEQ. ID. No. 1 as follows:

30 Met Ala Thr Ser Ser Thr Phe Ser Ser Leu Leu Pro Ser Pro Ala  
1 5 10 15

L u Leu Ser Asp His Arg Ser Pro Pro Pro Ser Ile Arg Tyr Ser Phe  
20 25 30

35 s r Pro Leu Thr Thr Pro Lys Ser Ser Arg Leu Gly Phe Thr Val Pro  
35 40 45

- 13 -

This protein, known as HrBP1p, is encoded by a cDNA molecule having SEQ. ID. No. 2 as follows:

50	tttttccttc	tcaacaatgg	cgacttcttc	tactttctcg	tcactactac	cttcaccacc	60
	agctcttctt	tccgaccacc	gttctcctcc	accatccatc	agatactcct	tttctccctt	120
	aactactcca	aaatcgtctc	gtttgggttt	cactgtaccg	gagaagagaa	acctcgctgc	180
	taattcgtct	ctcgttgaag	tatccattgg	cggagaaagt	gaccaccac	catcatcatc	240
	tggatcagga	ggagacgaca	agcaaattgc	attactcaaa	ctcaaattac	ttagtgtagt	300
55	ttcgggatta	aacagaggac	ttgtggcgag	tgttgatgat	ttagaaagag	ctgaagtggc	360
	tgctaaagaa	cttgaaactg	ctgggggacc	ggttgattta	accgatgata	ttgataagct	420
	tcaagggaaa	tggaggctgt	tgtatagtag	tgcgttctct	tctcggctct	tagtggttag	480
	ccgtcctggg	ctacctactg	gacgtttgat	ccctgttact	cttggccagg	tgtttcaacg	540

- 14 -

gattgatgtg tttagcaaag attttgataa catagcagag gtggaattag gagccccttg 600  
 gccatttccg ccattagaag ccactgcgac attggcacac aagtttgaac tcttaggcac 660  
 ttgcaagatc aagataacat ttgagaaaac aactgtgaag acatcgggaa acttgtcgca 720  
 gattcctccg tttgatatcc cgaggcttcc cgacagtttc agaccatcgt caaaccttg 780  
 5 aactggggat ttcgaagtta cctatgttga tgataccatg cgcataactc gcggggacag 840  
 aggtgaactt aggttattcg tcattgctta attctcaaag ctttgacatg taaagataaa 900  
 taaatacttt ctgcttgatg cagtctcatg agttttgtac aaatcatgtg aacatataaa 960  
 tgcgctttat aagtaaatga gtgtcttggt caatgaatca 1000

10 The genomic DNA molecule containing the receptor encoding cDNA  
 molecule of SEQ. ID. No. 2 has SEQ. ID. No. 3 as follows:

aattagaaaa attaacaacc aacatctagt tagaatatit aatttgcacc aatgtcttcg 60  
 agtatagtga aaaaaataga agatcgaata tcgaatagta cgtatagaat catctagatc 120  
 15 cattcgaact aacgtctact tttcttttcc agcattaaca tgtagcttgt cattagcatt 180  
 tacatgttgc aaataacaca aattgggaaa ttgaaagact aaaaaacctt gtacagcaga 240  
 tggtttaaca cgtggattca tggacacaaa cagaaaacgg cagaactaag cacaaaaacg 300  
 tcaactaagc atatcaaagc ttttaatgca agcctaatat aaacacaagt ggttatccat 360  
 aatctgttct taatctcttg cagtgttat cttttcatta ttcgcaattc gcaattctat 420  
 20 attcttatat ttcaacttgt tcttcttcca aattgtaatt atatctacat cgtcttagct 480  
 tgaccattat agctccagta ccaagttctc ttcttaactt taatatcagc tactattctc 540  
 atactgtaaa tatcttttgt tcaccaaaca tatatttcga accaaactgc taaaagctta 600  
 tcataaattg cagttctagc cacacaattt tgcagttcca accattaaat gccacaaaat 660  
 ttggaagatt tcttaagaca agaagaacat agcaaccaa ccttattgat taaatatgaa 720  
 25 atgtctccat aaaactggga gatttcccca aataaagaga acacggcaaa tgttcacgta 780  
 atctccaaga tgaatgttta attttttctt tcagaaaaaa acaaaaaaac ttaactcaat 840  
 atagacaact agaattgata ccaactaagc aaaagaaatt caaaagacaa atatatat 900  
 gatatgaagt tacattatit tcaaaactta tatactacta aaagcctaaa aatttgttct 960  
 aaaatgatat ccaaataaat ggaaggcatg aatgtcatat gactaaaaga gaaaaacaca 1020  
 30 cctgtatata agtattgat catgctgcct ccgagtgaca aaacatacga tgtgggtctt 1080  
 tattgggcca tacttaaatg gaaaaaggag aaaaaaaatt gggcaatgtc tatgggtcga 1140  
 atttatatgt tttacatcaa taaaatcaat atttaatttt atatatgtgg gtcttaatct 1200  
 agtattatct acatagatta aaatcaaagt actgcatatg gtccataata atacaacca 1260  
 agcaaattaa aattttgttg cacaaaacga catcatttta ctcagaaagt aatatgcaat 1320  
 35 ttcgttttacg cacacacgta tacgcgctaa taaccctggg tgcttctcaa atcacataat 1380  
 aattaaagtc ttcttcttct tcttcttctc tacaaattat ctactctctc tcgttttttt 1440  
 ttcttctc acaatggcga cttcttctac tttctcgtca ctactacct caccaccagc 1500  
 tcttctttcc gaccaccgtt ctctccacc atccatcaga tactcctttt ctcccttaac 1560  
 tactccaaaa tcgtctcgtt tgggtttcac tgtaccggag aagagaaacc tcgctgctaa 1620  
 40 ttcgtctctc gttgaagtat ccattggcgg agaaagtgac ccaccacat catcatctgg 1680  
 atcaggagga gacgacaagc aaattgcatt actcaaactc aaattacttg tgagtctgat 1740  
 tcaaaccaat cggtgaaatt ataagaaatt gggttctgtt ctttgggaatt aggttttata 1800  
 ttactgttaa gattcgatta tagagtgaat tttgggaaga tttttcagat ttgatttgtg 1860

- 15 -

atgtgttggtg ttgtgagaaa ttgcagagtg tagtttcggg attaaacaga ggacttggtg 1920  
cgagtgttga tgatttagaa agagctgaag tggctgctaa agaacttgaa actgctgggg 1980  
gaccggttga ttttaaccgat gatcttgata agcttcaagg gaaatggagg ctggtgtata 2040  
gtagtgcgtt ctcttctcgg tctttaggtg gtagccgtcc tggctctacct actggacgtt 2100  
5 tgatccctgt tactcttggc caggtaattc ttgaatcatt gctctgtttt taccgcgtaa 2160  
gattcggttt ttcgggtaag ttgttgagga gtttatgtgc atggtctagt ctatcatcaa 2220  
tagtcttgct tgagtttgaa tggggctgag ctaagaatct agctttctga ggttacaatt 2280  
tggaatgtc atcttatact cgaagcaaa cttttttcta tattgtcaag tttatgtgta 2340  
cggctctggtc tatcattggt agtctttggt gagcttgaat ggtgaggagc ttagaatcta 2400  
10 gcaatgtcat ctactcctta atcatttttt tctatattgc caagtttatg tgtacggctc 2460  
tagtcaatca tctttattct tggttgagtt tgaatggtga tgagcttaga atctagcttt 2520  
ctttggttta aatttggtgaa agaaccatac ctgaatcggg agaagcaaa cttctttaat 2580  
attatctctt gtttctgaat cattaaaaa ggtgtttcaa cggattgatg tgtttagcaa 2640  
agattttgat aacatagcag aggtggaatt aggagcccct tggccatttc cgccattaga 2700  
15 agccactgcy acattggcac acaagtttga actcttaggt ttgcatttcc ctttctctca 2760  
ttaagtttat cgaatttgtt aagagcaaaa taacttatct gtatctttga catttatggg 2820  
gaaaacaggc acttgcaaga tcaagataac atttgagaaa acaactgtga agacatcggg 2880  
aaacttgctg cagattcctc cgtttgatat cccgaggctt cccgacagtt tcagaccatc 2940  
gtcaaacctt ggaactgggg atttcgaagt tacctatggt gatgatacca tgcgcataac 3000  
20 tcgcggggac agaggtgaac ttagggattt cgtcattgct taattctcaa agctttgaca 3060  
tgtaaagata aataaatact ttctgcttga tgcagtctca tgagttttgt acaaatcatg 3120  
tgaacatata aatgcgcttt ataagtaaat gagtgtcttg ttcaatgaat catatgaaag 3180  
aatttgatg actcagaaaa ttggacaatg atatagacct tccaaatttt gcacctcta 3240  
atgtgagata ttagtgattt tttcttaggt tggtagagag aacggattgg caaaaaata 3300  
25 tcgaaggcca atgattaaca gcaaaacat atcttgatga ttcaaaatat agagttaaca 3360  
agcaaagatg agacaatctt atacgagaga gctaaaacaa atggattcca aatccagcaa 3420  
gtacaaaaat cgcagaaaat aagatgaaac caacttaaaa cagagatggt cccttccct 3480  
tcttgtcacc accgatctcg aaatgcttgc acctctgaaa taaacaacaa accaacacaa 3540  
tgtaagcaaa ttaccaagtt acaaatccgg tataatgaac tgatctatgt tctatgcacc 3600  
30 ttgataggac gctgcgaaaa gtgcttgag ctttgacact gaagcctcaa aacaatcttc 3660  
ttcgtggtct tagcctgtta acaagattca caagatgtat ctcaagtcaa aactgagact 3720  
attggaatgt ctgtttcctc acagctcact tccaaaatc tactataaat ggttccttaa 3780  
aactacctca tttcaactaa ctagacctaa ttcaaactga aaaaacaatc aatgcatgat 3840  
aatcaatgtt acctttttgt ggaagacagg cttagtctga ccaccataac cagattgttt 3900  
35 acggtcataa cgacgcttc cttgagcagc aagactgtct ttacccttct tgtattgggt 3960  
aaccttgctc aaagtatgct ttttgcatc cttgttctta cagtaagtgt tctttgtctt 4020  
tggaatgttc accttcaaaa ttcataaaat caaaaatgaa tcaactcacac acatacaaaa 4080  
tcaagagact ttttaaggta atcaaaatc aaacatcatt tagattgaaa acttttatga 4140  
tagatctgaa aaacaatata ataaatcaat caaccatgta ttgttgttct tcaaagtcaa 4200  
40 cgaactttac aaattccaaa atcacatcga aagagaagaa acaatttacc attttcgct 4260

- 16 -

Another example of a receptor in accordance with the present invention is that found in rice which has a partial amino acid sequence of SEQ. ID. No. 4 as follows:

```

5  Val Ala Ala Leu Lys Val Lys Leu Leu Ser Ala Val Ser Gly Leu Asn
    1           5           10           15
    Arg Gly Leu Ala Gly Ser Gln Glu Asp Leu Asp Arg Ala Asp Ala Ala
    20           25           30
10  Ala Arg Glu Leu Glu Ala Ala Ala Gly Gly Gly Pro Val Asp Leu Glu
    35           40           45
    Arg Asp Val Asp Lys Leu Gln Gly Arg Trp Arg Leu Val Tyr Ser Ser
    50           55           60
15  Ala Phe Ser Ser Arg Thr Leu Gly Gly Ser Arg Pro Gly Pro Pro Thr
    65           70           75           80
    Gly Arg Leu Leu Pro Ile Thr Leu Gly Gln Val Phe Gln Arg Ile Asp
    85           90           95
    Val Val Ser Lys Asp Phe Asp Asn Ile Val Asp Val Glu Leu Gly Ala
    100          105          110
25  Pro Trp Pro Leu Pro Pro Val Glu Leu Thr Ala Thr Leu Ala His Lys
    115          120          125
    Phe Glu Ile Ile Gly Thr Ser Ser Ile Lys Ile Thr Phe Asp Lys Thr
    130          135          140
    Thr Val Lys Thr Lys Gly Asn Leu Ser Gln Leu Pro Pro Leu Glu Val
    145          150          155          160
35  Pro Arg Ile Pro Asp Asn Leu Arg Pro Pro Ser Asn Thr Gly Ser Gly
    165          170          175
    Glu Phe Glu Val Thr Tyr Leu Asp Gly Asp Thr Arg Ile Thr Arg Gly
    180          185          190
40  Asp Arg Gly Glu Leu Arg Val Phe Val Ile Ser
    195          200

```

45 This protein, known as R6p, is encoded by a cDNA molecule which has a partial sequence corresponding to SEQ. ID. No. 5 as follows:

```

cgtggctgcg ctcaaagtca agcttctgag cgcggtgtcc gggctgaacc gcggcctcgc 60
50  ggggagccag gaggatcttg accgcgccga cgcggcggcg cgggagctcg aggcggcggc 120
    ggggtggcggc cccgtcgacc tggagaggga cgtggacaag ctgcaggggc ggtggaggct 180
    ggtgtacagc agcgcgttct cgtcgcggac gctcggcggc agccgccccg gcccgccac 240
    cggccgcctc ctcccatca ccctcgggca ggtgtttcag aggatcgatg ttgtcagcaa 300
    ggacttcgac aacatcgtcg atgtcgagct cggcgcgcca tggccgctgc cgcgggtgga 360
    gctgacggcg accctggctc acaagtttga gatcatcggc acctcgagca taaagatcac 420
55  attcgacaag acgacggtga agacgaaggg gaacctgtcc cagctgccgc cgctggaggt 480
    ccctcgcac cccgacaacc tccggccgcg gtccaacacc ggcagcggcg agttcgaggt 540
    gacctacctc gacggcgaca cccgcatcac ccgcggggac agaggggagc tcagggtgtt 600

```

cgtcatctcg tga

613

5 Hypersensitive response elicitors recognized by the receptors of the present invention are able to elicit local necrosis in plant tissue contacted by the elicitor.

Examples of suitable bacterial sources of hypersensitive response elicitor polypeptides or proteins include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*,  
10 *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*,  
15 *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* is disclosed in U.S. Patent No. 5,850,015 and U.S. Patent No. 6,001,959, which are hereby incorporated by reference. This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a  
20 glycine content of greater than 16%, and contains substantially no cysteine.

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has a glycine content of greater  
25 than 21% and contains substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in U.S. Patent No. 5,849,868 to Beer and Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which are hereby incorporated by reference.

30 The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* and its encoding

- 18 -

DNA molecule is found in U.S. Patent Nos. 5,708,139 and 5,858,786 and He et al., "Pseudomonas syringae pv. syringae Harpin<sub>PSS</sub>: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-66 (1993), which are hereby incorporated by reference.

5 The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is  
10 hereby incorporated by reference. This protein has 344 amino acids, a molecular weight of 33.2 kDa, and a pI of 4.16, is heat stable and glycine rich (20.6%).

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has a partial amino acid sequence corresponding to SEQ. ID. No. 6 as follows:

15 Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala  
1 5 10 15  
20 Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr  
20 25

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris*  
25 pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a  
30 molecular weight of 20 kDa. It has the amino acid sequence of SEQ. ID. No. 7 as follows:

Met Asp Ser Ile Gly Asn Asn Phe Ser Asn Ile Gly Asn Leu Gln Thr  
1 5 10 15  
35 Met Gly Ile Gly Pro Gln Gln His Glu Asp Ser Ser Gln Gln Ser Pro  
20 25 30  
Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln Leu Leu Ala Met Phe Ile  
35 40 45

40



	Met	Met	Met	Leu	Gln	Gln	Ser	Gln	Gly	Ser	Asp	Ala	Asn	Gln	Glu	Cys
	50						55					60				
5	Gly	Asn	Glu	Gln	Pro	Gln	Asn	Gly	Gln	Gln	Glu	Gly	Leu	Ser	Pro	Leu
	65					70					75					80
	Thr	Gln	Met	Leu	Met	Gln	Ile	Val	Met	Gln	Leu	Met	Gln	Asn	Gln	Gly
					85					90					95	
10	Gly	Ala	Gly	Met	Gly	Gly	Gly	Gly	Ser	Val	Asn	Ser	Ser	Leu	Gly	Gly
				100					105					110		
	Asn			Ala												
15																

20	atggactcta	tcggaacaa	cttttcgaat	atcggcaacc	tgcagacgat	gggcatcggg	60
	cctcagcaac	acgaggactc	cagccagcag	tgccttcgg	ctggctccga	gcagcagctg	120
	gatcagttgc	tgccatggt	catcatgatg	atgctgcaac	agagccaggg	cagcgatgca	180
	aatcaggagt	gtggcaacga	acaaccgcag	aacggtcaac	aggaaggcct	gagtccggtt	240
	acgcagatgc	tgatgcagat	cgtgatgcag	ctgatgcaga	accagggcgg	cgccggcatg	300
25	ggcggtgggc	gttcggtcaa	cagcagcctg	ggcggcaacg	cc		342

The hypersensitive response elicitor protein or polypeptide of *Erwinia* *stewartii* is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

40                      Hypersensitive response elicitor proteins or polypeptides from  
*Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*,  
*Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are

described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992), Baillreul et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference. These hypersensitive response elicitors from *Phytophthora* are called elicitins. All known elicitins have 98 amino acids and show >66% sequence identity. They can be classified into two groups, the basic elicitins and the acidic elicitins, based on the physicochemical properties. This classification also corresponds to differences in the elicitins' ability to elicit HR-like symptoms. Basic elicitins are 100 times more effective than the acidic ones in causing leaf necrosis on tobacco plants.

The hypersensitive response elicitor from Gram positive bacteria like *Clavibacter michiganensis* is described in WO 99/11133, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response using conditions under which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Turning again to the receptor of the present invention for such hypersensitive response elicitors, fragments of the above receptor protein are encompassed by the method of the present invention. In addition, fragments of full length receptor proteins from other plants can also be utilized.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known receptor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for receptor activity according to the procedure described above.

As an alternative, fragments of a receptor protein can be produced by digestion of a full-length receptor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave receptor proteins at different sites based on the amino acid sequence of the receptor protein. Some of the fragments that result from proteolysis may be active receptors.

In another approach, based on knowledge of the primary structure of the receptor protein, fragments of the receptor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the receptor being produced. Alternatively, subjecting a full length receptor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a tag or other sequence for ease of synthesis, purification, or identification of the polypeptide.

Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of 50 continuous bases of SEQ. ID. No. 2 under stringent conditions characterized by a hybridization buffer comprising 0.9M sodium

- 22 -

citrate ("SSC") buffer at a temperature of 37°C and remaining bound when subject to washing with the SSC buffer at 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

The receptor of the present invention is preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the receptor of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the receptor protein of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the receptor protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The cell lysate can be further purified by conventionally utilized chromatography procedures (e.g., gel filtration in an appropriately sized dextran or polyacrylamide column to separate the receptor protein). If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the receptor protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

- 23 -

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral  
5 vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET-series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes,"  
10 Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard  
15 cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host  
20 cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression  
25 elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).  
30

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from

- 24 -

those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

5                   Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG,  
10                   which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby  
15                   incorporated by reference.

                  Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a  
20                   number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P<sub>R</sub> and P<sub>L</sub> promotors of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA  
25                   segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

                  Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations,  
30                   the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG

- 25 -

(isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the receptor protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

One aspect of the present invention involves enhancing a plant's receptivity to treatment with a hypersensitive response elicitor by providing a transgenic plant or transgenic plant seed, transformed with a nucleic acid molecule encoding a receptor protein for a hypersensitive response elicitor. It has been found that hypersensitive response elicitors are useful in imparting disease resistance to plants, enhancing plant growth, effecting insect control and/or imparting stress resistance in a variety of plants. In view of the receptor of the present invention's interaction with such elicitors, it is expected that these beneficial effects would be enhanced by carrying out such elicitor treatments with plants transformed with the receptor encoding gene of the present invention.

- 26 -

Transgenic plants containing a gene encoding a receptor in accordance with the present invention can be prepared according to techniques well known in the art.

5 A vector containing the receptor encoding gene described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

10 Another approach to transforming plant cells with a gene is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby  
15 incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be  
20 surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies.  
25 Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are  
30 electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes



- 27 -

allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing

- 28 -

transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedures. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds.

These elicitor treatment methods can involve applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed transformed with a receptor gene in accordance with the present invention under conditions effective for the elicitor to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, to effect insect control, and/or to impart resistance to stress.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, to control insects, and/or to impart stress resistance in the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant

- 29 -

transformed with both a DNA molecule encoding a receptor in accordance with the present invention and with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein. The plant is grown under conditions effective to permit the DNA molecules to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart resistance to stress. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and a DNA molecule encoding a receptor can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit the DNA molecules to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart resistance to stress.

The embodiment where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seeds cells. In these embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The hypersensitive response elicitor treatment can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, control insects, and/or impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

- 30 -

With regard to the use of hypersensitive response elicitors in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased.

- 5 This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

- 10 The method of imparting pathogen resistance to plants is useful in imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*.

- Resistance, *inter alia*, to the following bacteria can also be imparted to plants *Pseudomonas solanacearum*; *Pseudomonas syringae* pv. *tabaci*; and *Xanthomonas*  
15 *campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi: *Fusarium oxysporum* and *Phytophthora infestans*.

- With regard to the use of the hypersensitive response elicitor protein or polypeptide to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins  
20 from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, there is significant economic benefit to growers. For example, early  
25 germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

- 30 The use of hypersensitive response elicitors for insect control encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by

feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants  
5 resulting from insect infection.

Elicitor treatment is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding-  
10 pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable  
15 production worldwide.

Hypersensitive response elicitor treatment is also useful in imparting resistance to plants against environmental stress. Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought,  
20 water, frost, cold temperature, high temperature, excessive light, and insufficient light), air pollution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO<sub>x</sub>, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients).

25 The application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and  
30 leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, or

injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with a hypersensitive response elicitor, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to  
5 produce plants. After plants have been propagated from seeds treated with an elicitor, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, to control insects on the plants, and/or to impart stress resistance.

The hypersensitive response elicitor polypeptide or protein can be  
10 applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the elicitor can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers  
15 include water, aqueous solutions, slurries, or dry powders.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

20 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. In addition, the hypersensitive response elicitor can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative technique involving the use of transgenic plants and  
25 transgenic seeds encoding a hypersensitive response elicitor encoding gene, a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such an elicitor are produced according to procedures well known in the art as described above.

In another embodiment, the present invention relates to a DNA  
30 construct which is an antisense nucleic acid molecule to a nucleic acid molecule encoding a receptor in plants for plant pathogen hypersensitive response elicitors. An example of such a construct would be an antisense DNA molecule of the DNA

- 33 -

molecule having the nucleotide sequence of SEQ. ID. Nos. 2 or 3. Alternatively, the DNA construct can have a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2 or 3 (or a portion thereof) and its complementary strand and is used to generate a single transcript with an inverted repeat (i.e. a double-stranded) RNA. This transcript as well as the above-discussed antisense nucleic acid molecule can be used to induce silencing of a nucleic acid molecule encoding a receptor for a hypersensitive response elicitor.

Sensing the hypersensitive response elicitor by the receptor is the very first step of the signal transduction pathway in plants which eventually leads to disease resistance, growth enhancement, insect control, and stress resistance. Silencing the receptor provides a powerful tool to find and study the downstream components of this pathway. Additionally, the receptor could be a negative regulator of such plant signal transduction pathway. Silencing of the receptor will impart to plants the ability to resist disease and stress, control insects, and enhance growth without hypersensitive response elicitor treatment.

## EXAMPLES

### Example 1 - Materials and Methods

The laboratory technique used in the following example is straight forward. All DNA manipulations described here followed conventional protocols (Sambrook et al., "Molecular Cloning: A Laboratory Manual," 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory (1989); Ausubel, et al., "Current Protocols in Molecular Biology," John Wiley (1987), which are hereby incorporated by reference). The plasmids and microorganisms described herein, used for making the present invention, were obtained from commercial sources, or from the authors of previous publications. Sequences were analyzed with Clone Manager 5 (Scientific & Educational Software, Durham, North Carolina).

Yeast strain L40 was grown in YPD or in different minimal synthetic dropout selection media at 30°C. *E.coli* strains DH5 $\alpha$  and HB101 were grown in LB at 37°C.

The yeast Two-Hybrid system is based on the fact that many eukaryotic transcription factors are composed of a physically separable, functionally independent DNA-binding domain (DNA-BD) and an activation domain (AD). Both the DNA-BD and the AD are required to activate a gene. When physically separated by recombinant DNA technology and expressed in the same host cell, the DNA-BD and the AD do not interact directly with each other and, thus, cannot activate the responsive gene (Ma, et al., "Converting a Eukaryotic Transcriptional Inhibitor into an Activator," Cell 55:443 (1988) and Brent, et al., "A Eukaryotic Transcriptional Activator Bearing the DNA Specificity of a Prokaryotic Repressor," Cell 43:729 (1985), which are hereby incorporated by reference). But if the DNA-BD and the AD are brought into close physical proximity in the promoter region, the transcriptional activation function will be restored. Therefore, the yeast *Saccharomyces cerevisiae* and the Two-Hybrid system have become essential genetic tools for studying the macromolecular interactions.

In the Two-Hybrid system utilized here, the DNA-BD, encoded in the bait vector pVJL11 (Jullien-Flores, V., "Bridging Ral GTPase to Rho Pathways. RLIP76, a Ral Effector with CDC42/Rac GTPase-activating Protein Activity," J. Biol. Chem. 27:22473 (1995), which is hereby incorporated by reference), is the prokaryotic LexA protein, and the activation domain, encoded in the prey vector pGAD 10 or pGAD GH (Clontech; Hannon, GJ., "Isolation of the Rb-related p130 Through its Interaction with CDK2 and Cyclins," Genes Dev. 7:2378 (1993), which is hereby incorporated by reference) is derived from the yeast GAL4 protein. pVJL11 also has a *TRP1* marker, and the pGAD a *LEU2* marker. An interaction between the bait protein (fused to the DNA-BD) and a library-encoded protein (fused to the AD) creates a novel transcriptional activator with binding affinity for LexA operators. The yeast host L40 {MATa his3D200 trp1-901 leu2-3, 112 ade2 LYS2::(lexAop)<sub>4</sub>-HIS3 URA3::(lexAop)<sub>8</sub>-lacZ} harbors two reporter genes, *lacZ* and *HIS3*, which contain upstream LexA binding site. The *HIS3* nutritional reporter provides a sensitive growth selection that can identify a single positive transformant out of several million candidate clones. The expression of the reporter genes indicates interaction between a candidate protein and the bait protein. See Figure 1.



*Erwinia amylovora* harpin was used as the bait protein to screen the *Arabidopsis thaliana* MATCHMAKER cDNA library cloned in the pGAD 10 vector (Clontech Laboratories, Inc., Palo Alto, California). One cDNA library encoded protein was identified as a strong harpin interacting protein and, thus, a putative harpin receptor. The present invention reports the nucleic acid sequence and the deduced amino acid sequence of this cDNA.

### Example 2

HrpN of *Erwinia amylovora* was subcloned into the yeast Two-Hybrid bait vector pVJL11. PCR was carried out using the 1.3 Kb harpin fragment (Wei et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992), which is hereby incorporated by reference) as a template to amplify the harpin encoding region. A Bam HI site was added to the 5' end of the coding sequence, and a Sal I site to the 3' end. A Bam HI and Sal I digested PCR fragment was ligated with the bait vector pVJL11 digested with the same restriction enzymes. pVJL11 has a *TRP1* marker to be selected in yeast and an Amp resistance marker to be selected in *E. coli*. The plasmid DNA was amplified in *E. coli* strain DH5 $\alpha$ . When tested in the Two-Hybrid system with empty prey vector pGAD GH and several unrelated proteins, HrpN didn't show auto-activation or nonspecific interaction with unrelated proteins, as shown in Figure 2.

### Example 3

HrpN-pVJL 11 was transformed into yeast strain L40 by a lithium acetate (LiAc)-mediated method (Ito et al., "Transformation of Intact Yeast Cells Treated with Alkali Cations," J. Bacteriol. 153:163 (1983) and Vojtek et al., "Mammalian Ras Interacts Directly with the Serine/Threonine Kinase Raf," Cell 74:205 (1993), which are hereby incorporated by reference). The *Arabidopsis thaliana* MATCHMAKER cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) was screened for harpin interacting proteins. Approximately 6.8 million primary library transformants were plated onto plates lacking histidine, leucine, and tryptophan. A total of 148 colonies grew on the histidine dropout plates, 55 of which

stained positive when tested for expression of  $\beta$ -galactosidase. After three rounds of selection on synthetic minimal (SD) media plates lacking leucine, tryptophan, and histidine, and confirming by the expression of the second reporter gene *lacZ* using a  $\beta$ -galactosidase assay, 47 colonies seemed to be strong interacting candidates.

5

#### **Example 4**

Plasmid DNA was extracted from the 47 independent yeast colonies and shuttled into *E. coli* strain HB101, which carries the *leuB* mutation. Therefore, the prey plasmid (cDNA-pGAD 10) was selected for on minimal nutrient plates since pGAD 10 bears the *LEU2* marker.

The 47 independently rescued prey plasmids purified from *E. coli* were re-tested in the yeast two-hybrid system with harpin as bait. They were also tested against unrelated proteins. 25 turned out to be interacting candidates, 20 of which were strong specific interacting candidates. Sequencing analysis showed that the 20 independent cDNA clones were actually from the same gene with different integrity at the 5' end. The sequence reactions were performed using the PE Prism BigDye™ dye terminator reaction kit. The sequencing gel was run in Thatagen (Bothell, WA)

One of the eight plasmids, which had the longest cDNA insert of 1kb, was used for further analysis. When co-transformed into yeast strain L40, it was shown to be negative with empty bait and unrelated proteins in the Two-Hybrid system, indicating the specificity of the interaction between harpin and this receptor candidate. See Figure 3.

#### **Example 5**

The longest cDNA insert, HrBP1, was subcloned into the Bam HI and SalI sites of the bait vector pVJL 11. This construct didn't show auto-activation of the reporter genes, nor interaction with unrelated proteins in the yeast Two-Hybrid system. However, the expression of the reporter genes was activated when L40 was co-transformed with HrBP1-pVJL11 and HrpN-pGAD GH, indicating the specific interaction between HrBP1p (the protein encoded by HrBP1) and harpin. See Figure 4.

### Example 6

5 Total RNA was extracted from two-week-old *Arabidopsis thaliana* using QIAGEN RNeasy plant mini kit (Qiagen, Inc., Valencia, California). Poly A<sup>+</sup> RNA was further purified from the total RNA with a QIAGEN Oligotex column (Qiagen, Inc., Valencia, California). A Northern blot was carried out using the translated region of HrBP1 as a probe. One single species with an apparent molecular weight of about 1.1 Kb was detected from both total RNA and Poly A<sup>+</sup> RNA.

10 Therefore, the longest cDNA of HrBP1 from the yeast two-hybrid screen seems to be the full-length cDNA. The integrity of the 5' of cDNA was further confirmed by a primer extension assay.

As described, a yeast Two-Hybrid system was used to screen for harpin interacting proteins. HrpN of *Erwinia amylovora* was subcloned into the yeast  
15 Two-Hybrid bait vector pVJL11, which has a *TRP1* marker. The lexA harpin fusion protein is expressed from this construct in yeast. The *Arabidopsis thaliana* MATCHMAKER cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) was screened for hypersensitive response elicitor interacting proteins. 6.8 million independent colonies were screened, and HrBP1 was identified as a strong specific  
20 harpin interacting candidate. HrBP1 was mapped to *Arabidopsis thaliana* genomic DNA, chromosome 3, P1 clone MLM24 (Nakamura, "Structural Analysis of *Arabidopsis thaliana* chromosome 3," Direct submission to the DDBJ/EMBL/GenBank databases (1998), which is hereby incorporated by reference). Four exons and three introns were discovered (See Figure 5). Exon 4 includes a 130  
25 bp non-translated 3' region. The in-frame open reading frame from the first methionine encodes a polypeptide (named HrBP1p) of 284 amino acids. The predicted molecular weight of HrBP1p is 30454.3 and pI is 5.72. There is no apparent hydrophobic trans-membrane domain in this polypeptide. SMART Simple Modular Architecture Research Tool (V3.1) predicted the first 18 amino acids as a signal  
30 sequence. The HrBP1-AD fusion prey was negative with empty bait and unrelated proteins in the yeast 2-H system, indicating the specificity of the interaction between harpin and this receptor candidate. When being put in the opposite orientation, i.e.

- 38 -

HrBP1p fused with the DNA-BD and harpin with the AD, they still specifically interacted with each other.

HrBP1 has no significant sequence similarity with sequences deposited in major sequence database accessible with the Blast search program. Therefore,  
5 HrBP1p is a novel protein.

### Example 7

The HrBP1 cDNA was subcloned into the Nde I and Sal I sites of the  
10 vector pET-28a (Novagen, Madison, WI). HrBP1p was expressed from this vector in *E. coli* as a His-tagged protein and purified with Ni-NTA resin (QIAGEN Inc., Valencia, CA) according to the manual provided by the manufacturer. This recombinant protein increased harpin's ability to induce HR in tobacco plants. His-tag removed HrBP1 recombinant protein was used to generate anti-HrBP1 antibody to  
15 facilitate biochemical and functional studies of HrBP1. Preliminary localization study using anti-HrBP1 antibody in a Western blot showed that HrBP1p exists everywhere in *Arabidopsis*, including its leaves, stems, and roots.

### Example 8

20 10µg of total RNA from 14 different plant species was separated on 1% agarose gel, and then transferred to Amersham Hybond NX membrane (Amersham Pharmacia Biotech, Piscataway, New Jersey). The RNA probe, which was complementary to bases 651-855 of HrBP1 coding region, was generated using  
25 Ambion Strip-EZ RNA kit (Ambion Inc., Houston, Texas). Membrane hybridization was done with Ambion ULTRAhyb (Ambion Inc., Houston, Texas), procedure according to manufacturer recommendation.

The sequence of the HrBP1 fragment used to generate the Northern probe (SEQ. ID. No. 9) is as follows:

30 gatcaagata acatttgaga aaacaactgt gaagacatcg ggaaacttgt cgcagattcc 60  
tccgtttgat atcccgaggc ttcccagacag ttccagacca tcgtcaaacc ctggaactgg 120  
ggatttcgaa gttacctatg ttgatgatac catgcgcata actcgcgggg acagagggtga 180  
acttagggta ttcgtcattg cttaa 205  
35

- 39 -

This Northern blot picked up a band with similar size as HrBP1 in all the plant species tested, including tobacco, wheat, corn, citrus, cotton, grass, pansy, pepper, potato, tomato, soybean, sun flower, and lima bean, which indicated HrBP1-like genes exist universally. See Figure 6.

5

**Example 9**

HrBP1 homologue from rice, R6, was clone by yeast two-hybrid screening using harpin as bait. It not only interacted with full length harpin but also  
 10 interacted with a harpin fragment that contains the second HR domain (see Figure 7). However, it is not a full-length cDNA; there is some sequence information missing from the 5' end. The partial sequence of HrBP1-like cDNA from rice encodes a peptide of 203 amino acids, R6-p, which starts at amino acid 84 of HrBP1p. They are 74.4% identical and 87.2% positive at the protein level, they are 65% identical at the  
 15 DNA level.

The following shows the sequence alignment of HrBP1 (SEQ. ID. No. 1 starting at amino acid 84) and R6 (SEQ. ID. No. 4) at the protein level:

At protein level: Identities = 151/203 (74.4%), Positives = 177/203 (87.2%), Gaps = 2/203 (0%)

20 R6-p : 1 VAALKVKLLSAVSGINRGLAGSQEDLDRADAAARELEAAAGGGPVDLERDVKLQGRWRL  
 +A LK+KLLS VSGLNRGL S +DL+RA+ AA+ELE A GGPVDL D+DKLQG+WRL  
 HrBP1p: 84 IALLKLLKLLSVVSGINRGLVASVDDLERAEVAAKELETA--GGPVDLTDDLDKLGKWRL

25 R6-p : 61 VYSSAFSSRTLGGSRPGPPTGRLLPITLGQVFQRIDVVS KD FDNIVDVELGAPWPLPPVE  
 +YSSAFSSR+LGGSRPG PTGRL+P+TLGQVFQRIDV SKDFDNI +VELGAPWP PP+E  
 HrBP1p: 142 LYSSAFSSRSLGGSRPGLPTGRLLPVTLGQVFQRIDVFSKDFDNIAEVELGAPWFPFPLE

30 R6-p : 121 LTATLAHKFEIIGTSSIKITFDKTTVKTKGNLSQLPPLEVRIPDNLRPPSNTGSGEFEV  
 TATLAHKFE++GT IKITF+KTTVKT GNLSQ+PP ++PR+PD+ RP SN G+G+FEV  
 HrBP1p: 202 ATATLAHKFELLGTCKIKITFEKTTVKTS GNLSQI PPFDIPRLPDSFRPSSNPGTGDFEV

R6-p : 181 TYLDGDTTRITRGDRGELRVFVIS 203  
 TY+D RITRGDRGELRVFI+

35 HrBP1p: 262 TYVDDTMRITRGDRGELRVFVIA 284

The sequence alignment, on a DNA level, of R6 (SEQ. ID. No. 5) and HrBP1 (SEQ. ID. No. 2) starting at nucleotide 265 (i.e. nucleotide 249 of the open reading frame)

40

At DNA level: Identities = 397/610 (65%) (dots indicate identical bases)

45 R6 1 cgtggctgctgctcaaagtcaagcttctgagcgcggtgtccgggctgaaccgcgccctcgc  
 HrBP1 249 aa.t..atta.....c....at.a..t..t.ta..t..g..at.a...a.a...t.t

*Arabidopsis thaliana* Columbia plants were grown in autoclaved  
35 potting mix in a controlled environment room at a day and night temperature of  
23-20°C and a photoperiod of 14 h light.

*Arabidopsis* plants were also transformed with a construct, which has an inverted repeat with a sense strand of HrBP1 coding region bases 4-650 (i.e. bases 20-666 of SEC. ID. No. 2) and the complementary sequence of bases 20-516 of HrBP1 cDNA (i.e. SEQ. ID. No. 2). This construct generated a double-stranded mRNA in transformed plants. These transgenic lines were designated "d" lines.

Figure 8 shows the constructs used to transform *Arabidopsis*.

Both antisense and double-stranded approaches were to silence the expression of HrBP1. The double stranded RNA method was found to be more efficient in silencing the HrBP1 gene. Some transgenic *Arabidopsis* lines showed spontaneous HR-mimic lesion. The most severe line was developmentally retarded, looked very sick, and did not produce seeds.

### Example 11

Plants were grown in autoclaved potting mix in a controlled environment room with a day and night temperature of 23-20°C and a photoperiod of 14 h light. 25-day-old plants were inoculated with *Pseudomonas syringae* p.v. tomato DC3000 by dipping the above soil parts of the plants in  $10^8$  cells  $\text{ml}^{-1}$  bacteria suspension for 10 second. Seven days after DC3000 inoculation, leaf disks were harvested with cork borer. Bacteria were extracted from leaf disk in 10mM  $\text{MgCl}_2$  and plated on King's B agar containing 100  $\mu\text{g/ml}$  rifampicin. Plates were incubated at 28°C for 2 days (Figure 9B) and colonies counted. In Figure 9A, wild type *Arabidopsis* plants had significantly more disease development than transgenic plants. Bacteria counting (Figure 9C) showed that transgenic plants had at least one magnitude less of DC3000 growing inside the leaves. HrBP1 seemed like a negative regulator of plant defense signal transduction pathway in *Arabidopsis*. Its silencing imparted plants with the ability to resist *Pseudomonas syringae* p.v. tomato DC3000.

### Example 12

HrBP1 coding region, bases 17-871 of SEQ. ID. No. 2, was sub-cloned into binary vector pPZP212 which is under the control of the NOS promoter (see Figure 10). Tobacco plants were transformed with this construct via an *Agrobacteria* mediated method. The *Agrobacterium tumefaciens* strain used was LBA4404.

### Example 13

HrBP1 was over-expressed in tobacco plants under the control of an NOS promoter. Figure 10 shows the construct used for tobacco transformation. Three high expression lines were chosen for further studies in the T2 generation.

- 42 -

When infiltrated with purified harpin, the transgenic lines developed HR much faster than wild type plants, which is consistent with previous experiment in which His-tagged HrBP1 increased tobacco's sensitivity to harpin protein. The HrBP1 over-expressing lines were about 20-30% taller than wild type Xanthi NN plants (see Figure 11).

#### **Example 14**

61-day-old wild type and HrBP1 over-expressing Xanthi NN tobacco plants were inoculated with tobacco mosaic virus by rubbing TMV with diatomaceous earth on the upper surface of leaves. Lesions appeared 2 days after manual inoculation. The picture in Figure 12A was taken 3 days after inoculation. The diameter of disease spots was measured. On average, the diameter of lesions on transgenic plant leaves were 33.4% less than that on wild type plants (Figure 12B). Therefore, the surface area of lesions on transgenic plant leaves was about 44.3% of those of the wild type plants. HrBP1 seemed to be a positive regulator of the plant signal transduction pathway for growth and disease resistance in tobacco.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.



- 43 -

**WHAT IS CLAIMED:**

1. An isolated protein which serves as a receptor in plants for plant pathogen hypersensitive response elicitors.  
5
2. A protein according to claim 1, wherein the plant pathogen is selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthamonas*, *Phytophthora*, and *Clavibacter*.
- 10 3. A protein according to claim 2, wherein the plant pathogen is an *Erwinia* pathogen.
4. A protein according to claim 3, wherein the plant pathogen is *Erwinia amylovora*.  
15
5. A protein according to claim 1, wherein the protein is from a monocot.
6. A protein according to claim 5, wherein the protein is from  
20 rice.
7. A protein according to claim 1, wherein the protein has a partial amino acid sequence of SEQ. ID. No. 4.
- 25 8. A protein according to claim 1, wherein the protein is from a dicot.
9. A protein according to claim 8, wherein the protein is from *Arabidopsis thaliana*.  
30
10. A protein according to claim 1, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

11. A protein according to claim 1, wherein the protein is recombinant.
- 5 12. An isolated nucleic acid molecule encoding a protein according to claim 1.
- 10 13. A nucleic acid molecule according to claim 12, wherein the plant pathogen is selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthamonas*, *Phytophthora*, and *Clavibacter*.
14. A nucleic acid molecule according to claim 13, wherein the plant pathogen is an *Erwinia* pathogen.
- 15 15. A nucleic acid molecule according to claim 14, wherein the plant pathogen is *Erwinia amylovora*.
16. A nucleic acid molecule according to claim 12, wherein the protein is from a monocot.
- 20 17. A nucleic acid molecule according to claim 16, wherein the protein is from rice.
18. A nucleic acid molecule according to claim 12, wherein the protein has a partial amino acid sequence of SEQ. ID. No. 4.
- 25 19. A nucleic acid molecule according to claim 12, wherein the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C.
- 30

- 45 -

20. A nucleic acid molecule according to claim 12, wherein the nucleic acid has a nucleotide sequence comprising SEQ. ID. No. 5.

5 21. A nucleic acid molecule according to claim 12, wherein the protein is from a dicot.

22. A nucleic acid molecule according to claim 21, wherein the protein is from *Arabidopsis thaliana*.

10 23. A nucleic acid molecule according to claim 12, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

15 24. A nucleic acid molecule according to claim 12, wherein the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C.

20 25. A nucleic acid molecule according to claim 12, wherein the nucleic acid has a nucleotide sequence of SEQ. ID. No. 2.

26. A nucleic acid according to claim 12, wherein the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C.

25 27. A nucleic acid according to claim 12, wherein the nucleic acid has a nucleotide sequence comprising SEQ. ID. No. 3.

30 28. An antisense nucleic acid molecule to the nucleic acid according to claim 12.

- 46 -

29. An expression vector containing a nucleic acid molecule according to claim 12 which is heterologous to the expression vector.

5 30. An expression vector according to claim 29, wherein the nucleic acid molecule is positioned in the expression vector in sense orientation and correct reading frame.

31. An expression vector according to claim 29, wherein either:  
10 (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising  
15 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature  
20 of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

32. An expression vector containing a nucleic acid molecule according to claim 28 which is heterologous to the expression vector.

25 33. A transgenic host cell transformed with the nucleic acid molecule according to claim 12.

34. A host cell transformed according to claim 33, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.

30

35. A host cell according to claim 33, wherein the DNA molecule is transformed with an expression system.

36. A host cell according to claim 33, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

37. A host cell transformed with a nucleic acid molecule according to claim 28.

38. A transgenic plant transformed with the DNA molecule of claim 12.

39. A transgenic plant according to claim 38, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

40. A transgenic plant according to claim 38, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

41. A transgenic plant according to claim 38, wherein the plant is a monocot.
- 5 42. A transgenic plant according to claim 38, wherein the plant is from a dicot.
- 10 43. A transgenic plant according to claim 38, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.
- 15 20
44. A transgenic plant transformed with a nucleic acid molecule according to claim 28.
- 25 45. A transgenic plant seed transformed with the DNA molecule of claim 12.
- 30 46. A transgenic plant seed according to claim 45, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber,

- 49 -

apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

47. A transgenic plant seed according to claim 45, wherein the  
5 plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*,  
petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

48. A transgenic plant seed according to claim 45, wherein the  
10 plant is a monocot.

49. A transgenic plant seed according to claim 45, wherein the  
plant is a dicot.

50. A transgenic plant seed according to claim 45, wherein either:  
15 (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid  
hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent  
conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M  
SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide  
sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence  
20 of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising  
20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the  
nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has  
an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the  
nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization  
25 buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature  
of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

51. A transgenic plant seed transformed with a nucleic acid  
molecule according to claim 28.  
30

52. A method of identifying agents targeting plant cells  
comprising:

- 50 -

forming a reaction mixture comprising a protein according to claim 1 and a candidate agent;  
evaluating the reaction mixture for binding between the protein and the candidate agent; and  
5 identifying candidate compounds which bind to the protein in the reaction mixture as plant cell targeting agents.

53. A method according to claim 52, wherein the protein is from a monocot.

10 54. A method according to claim 53, wherein the protein is from rice.

55. A method according to claim 52, wherein the protein has an amino acid sequence comprises SEQ. ID. No. 4.

56. A method according to claim 52, wherein the protein is from a dicot.

20 57. A method according to claim 56, wherein the protein is from *Arabidopsis thaliana*.

58. A method according to claim 52, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

25 59. A method of identifying agents targeting plant cells comprising:

forming a reaction mixture comprising a host cell transformed with a nucleic acid molecule according to claim 12 and a candidate agent;  
30 evaluating the reaction mixture for binding between protein produced by the host cell and the candidate agent; and



- 51 -

identifying candidate compounds which bind to the protein produced by the host cell in the reaction mixture as plant cell targeting agents.

5           60.    A method according to claim 59, wherein the protein is from a monocot.

          61.    A method according to claim 60, wherein the protein is from rice.

10           62.    A method according to claim 59, wherein the protein is from a dicot.

          63.    A method according to claim 62, wherein the protein is from *Arabidopsis thaliana*.

15           64.    A method according to claim 59, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

30           65.    A method of enhancing plant receptivity to treatment with hypersensitive response elicitors comprising:

          providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule according to claim 12.

66. A method according to claim 65, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

15

67. A method according to claim 65, wherein a transgenic plant is provided.

68. A method according to claim 65, wherein a transgenic plant seed is provided and said method further comprises:  
planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

69. A method according to claim 65, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

30

- 53 -

70. A method according to claim 65, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

5 71. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for imparting disease resistance.

72. A method according to claim 65, wherein the hypersensitive  
10 response elicitor treatment is for enhancing plant growth.

73. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for controlling insects.

74. A method according to claim 65, wherein the hypersensitive  
15 response elicitor treatment is for imparting stress tolerance.

75. A method according to claim 65, wherein the transgenic plant or plant seed is further transformed with a second nucleic acid encoding a hypersensitive response elicitor, wherein expression of the second nucleic acid effects  
20 the hypersensitive response elicitor treatment.

76. A method according to claim 65, wherein the hypersensitive response elicitor treatment comprises:  
25 applying a hypersensitive response elicitor to the plant or plant seed.

77. A method according to claim 76, wherein the hypersensitive response elicitor is applied in isolated form.

30 78. A method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants comprising:

providing a transgenic plant or transgenic plant seed transformed with a DNA construct effective to silence expression of a nucleic acid molecule according to claim 12.

5                   79.    A method according to claim 78, wherein the protein is from a monocot.

                  80.    A method according to claim 79, wherein the protein is from rice.

10                  81.    A method according to claim 78, wherein the protein is from a dicot.

                  82.    A method according to claim 81, wherein the protein is from *Arabidopsis thaliana*.

                  83.    A method according to claim 78, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

30                  84.    A method according to claim 78, wherein a transgenic plant is provided.

85. A method according to claim 78, wherein a transgenic plant seed is provided and said method further comprises:

planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

5

86. A method according to claim 78, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, 10 garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

87. A method according to claim 78, wherein the plant is selected 15 from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

88. A method according to claim 78, wherein the transgenic plant or plant seed is further transformed with a second nucleic acid encoding a 20 hypersensitive response elicitor, wherein expression of the second nucleic acid effects a hypersensitive response elicitor treatment.

89. A method according to claim 78 further comprising:  
applying a hypersensitive response elicitor to the plant or plant 25 seed.

90. A method according to claim 89, wherein the hypersensitive response elicitor is applied in isolated form.

30 91. A method according to claim 78, wherein disease resistance is imparted to plants.

- 56 -

92. A method according to claim 78, wherein enhanced growth is imparted to plants.

93. A method according to claim 78, wherein insect control is imparted to plants.

94. A method according to claim 78, wherein stress resistance is imparted to plants.

95. A method according to claim 78, wherein the DNA construct is an antisense nucleic acid molecule to a nucleic acid molecule encoding a receptor in plants for plant pathogen hypersensitive response elicitors.

96. A method according to claim 78, wherein the DNA construct is transcribable to a first nucleic acid encoding a receptor in plants for plant pathogen hypersensitive response elicitors coupled to a second nucleic acid encoding the inverted complement of the first nucleic acid.

97. A method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants comprising:  
providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule according to claim 12.

98. A method according to claim 97, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid

- 57 -

sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

5

99. A method according to claim 97, wherein a transgenic plant is provided.

100. A method according to claim 97, wherein a transgenic plant seed is provided and said method further comprises:  
planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

101. A method according to claim 97, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

20

102. A method according to claim 97, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

25

103. A method according to claim 97, wherein disease resistance is imparted.

104. A method according to claim 97, wherein plant growth is enhanced.

30

- 58 -

105. A method according to claim 97, wherein insects are  
controlled.
106. A method according to claim 97, wherein stress tolerance is  
5 imparted.
107. A method according to claim 97, wherein the protein is from a  
monocot.
108. A method according to claim 107, wherein the protein is from  
10 rice.
109. A method according to claim 97, wherein the protein is from a  
dicot.
110. A method according to claim 109, wherein the protein is from  
15 *Arabidopsis thaliana*.



## SEQUENCE LISTING

&lt;110&gt; Eden Bioscience Corporation

<120> RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS AND  
USES THEREOF

&lt;130&gt; 21829/63

&lt;140&gt;

&lt;141&gt;

&lt;150&gt; 60/191,649

&lt;151&gt; 2000-03-23

&lt;150&gt; 60/250,710

&lt;151&gt; 2000-12-01

&lt;160&gt; 9

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 284

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 1

Met Ala Thr Ser Ser Thr Phe Ser Ser Leu Leu Pro Ser Pro Pro Ala

1

5

10

15

Leu Leu Ser Asp His Arg Ser Pro Pro Pro Ser Ile Arg Tyr Ser Phe

20

25

30

Ser Pro Leu Thr Thr Pro Lys Ser Ser Arg Leu Gly Phe Thr Val Pro

35

40

45

Glu Lys Arg Asn Leu Ala Ala Asn Ser Ser Leu Val Glu Val Ser Ile

50

55

60

Gly Gly Glu Ser Asp Pro Pro Pro Ser Ser Ser Gly Ser Gly Gly Asp

65

70

75

80

Asp Lys Gln Ile Ala Leu Leu Lys Leu Lys Leu Leu Ser Val Val Ser

85

90

95

Gly Leu Asn Arg Gly Leu Val Ala Ser Val Asp Asp Leu Glu Arg Ala

100

105

110

Glu Val Ala Ala Lys Glu Leu Glu Thr Ala Gly Gly Pro Val Asp Leu  
 115 120 125  
 Thr Asp Asp Leu Asp Lys Leu Gln Gly Lys Trp Arg Leu Leu Tyr Ser  
 130 135 140  
 Ser Ala Phe Ser Ser Arg Ser Leu Gly Gly Ser Arg Pro Gly Leu Pro  
 145 150 155 160  
 Thr Gly Arg Leu Ile Pro Val Thr Leu Gly Gln Val Phe Gln Arg Ile  
 165 170 175  
 Asp Val Phe Ser Lys Asp Phe Asp Asn Ile Ala Glu Val Glu Leu Gly  
 180 185 190  
 Ala Pro Trp Pro Phe Pro Pro Leu Glu Ala Thr Ala Thr Leu Ala His  
 195 200 205  
 Lys Phe Glu Leu Leu Gly Thr Cys Lys Ile Lys Ile Thr Phe Glu Lys  
 210 215 220  
 Thr Thr Val Lys Thr Ser Gly Asn Leu Ser Gln Ile Pro Pro Phe Asp  
 225 230 235 240  
 Ile Pro Arg Leu Pro Asp Ser Phe Arg Pro Ser Ser Asn Pro Gly Thr  
 245 250 255  
 Gly Asp Phe Glu Val Thr Tyr Val Asp Asp Thr Met Arg Ile Thr Arg  
 260 265 270  
 Gly Asp Arg Gly Glu Leu Arg Val Phe Val Ile Ala  
 275 280

&lt;210&gt; 2

&lt;211&gt; 1000

&lt;212&gt; DNA

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 2

tttttccttc tcaacaatgg cgacttcttc tactttctcg tcactactac cttcaccacc 60  
 agctcttctt tccgaccacc gttctcctcc accatccatc agatactcct tttctccctt 120  
 aactactcca aaatcgtctc gtttggggtt cactgtaccg gagaagagaa acctcgctgc 180  
 taattcgtct ctcgttgaag tatccattgg cggagaaagt gaccaccac catcatcatc 240  
 tggatcagga ggagacgaca agcaaattgc attactcaaa ctcaaattac ttagtgtagt 300  
 ttcgggatta aacagaggac ttgtggcgag tgttgatgat ttagaaagag ctgaagtggc 360  
 tgctaaagaa cttgaaactg ctgggggacc ggttgattta accgatgatc ttgataagct 420

```

tcaagggaaa tggaggctgt tgtatagtag tgcgttctct tctcgggtctt taggtggttag 480
ccgtcctggg ctacctactg gacgtttgat ccctgttact cttggccagg tgtttcaacg 540
gattgatgtg tttagcaaag attttgataa catagcagag gtggaattag gagccccttg 600
gccatttccg ccattagaag ccactgcgac attggcacac aagtttgaac tcttaggcac 660
ttgcaagatc aagataacat ttgagaaaac aactgtgaag acatcgggaa acttgctcgca 720
gattcctccg tttgatatcc cgagggttcc cgacagtttc agaccatcgt caaaccttg 780
aactggggat ttcgaagtta cctatgttga tgataccatg cgcataactc gcggggacag 840
aggtgaactt aggggtattcg tcattgctta attctcaaag ctttgacatg taaagataaa 900
taaatacttt ctgcttgatg cagtctcatg agttttgtac aaatcatgtg aacatataaa 960
tgcgctttat aagtaaatga gtgtcttggt caatgaatca 1000

```

&lt;210&gt; 3

&lt;211&gt; 4260

&lt;212&gt; DNA

<213> *Arabidopsis thaliana*

&lt;400&gt; 3

```

aattagaaaa attaacaacc aacatctagt tagaatattt aatttgcacc aatgtcttcg 60
agtatagtga aaaaaataga agatcgaata tcgaatagta cgtatagaat catctagatc 120
cattcgaact aacgtctact tttcttttcc agcattaaca tgtagcttgt cattagcatt 180
tacatgttgc aaataacaca aattgggaaa ttgaaagact aaaaaacctt gtacagcaga 240
tggtttaaca cgtggattca tggacacaaa cagaaaacgg cagaactaag cacaaaaacg 300
tcaactaagc atatcaaagc ttttaatgca agcctaatat aaacacaagt gggttatccat 360
aatctgttct taatctcttg cagtagttat cttttcatta ttcgcaattc gcaattctat 420
attcttatat ttcaacttgt tcttcttcca aattgtaatt atatctacat cgtcttagct 480
tgaccattat agctccagta ccaagttctc ttcttaactt taatatcagc tactattctc 540
atactgtaaa tatcttttgt tcaccaaaca tatatttcga accaaaactgc taaaagctta 600
tcataaattg cagttctagc cacacaattt tgcagttcca accattaaat gccacaaaat 660
ttggacgatt tottaagaca agaagaacat agcaaccaa ccttattgat taaatatgaa 720
atgtctccat aaaactggga gatttcccca aataaagaga acacggcaaa tggtcacgta 780
atctccaaga tgaatgttta attttttctt tcagaaaaaa acaaaaaaac ttaactcaat 840
atagacaact agaatggata ccaactaagc aaaagaaatt caaaagacaa atatatttg 900
gatatgaagt tacattattt tcaaacttta tatactacta aaagcctaaa aatttgttct 960
aaaatgatat ccaaataaat ggaaggcatg aatgtcatat gactaaaaga gaaaaacaca 1020
cctgtatata agtattggat catgctgcct ccgagtgaac aaacatacga tgtgggtctt 1080
tattgggcca tacttaaatg gaaaaaggag aaaaaaaatt gggcaatgtc tatgggtcgaa 1140
atztatatgt tttacatcaa taaaatcaat atttaatttt atatatgtgg gtcttaatct 1200
agtattatct acatagatta aaatcaaagt actgcatatg gtccataata atacaaccaa 1260
agcaaattaa aattttgtgg cacaaaacga catcatttta ctacagaaagt aatatgcaat 1320
ttcgtttacg cacacacgta tacgcgctaa taaccggtgg tgcttctcaa atcacataat 1380
aattaaagtc ttcttcttct tcttcttctc tacaaattat ctactctctc tcgttttttt 1440
ttccttctca acaatggcga cttcttctac tttctcgtca ctactacctt caccaccagc 1500
tcttctttcc gaccaccgtt ctctccacc atccatcaga tactcctttt ctcccttaac 1560
tactccaaaa tcgtctcgtt tgggtttcac tgtaccggag aagagaaacc tcgctgctaa 1620
ttcgtctctc gttgaagtat ccattggcgg agaaagtgaac ccaccaccat catcatctgg 1680
atcaggagga gacgacaagc aaattgcatt actcaaactc aaattacttg tgagtctgat 1740
tcaaaccaat cgggtgaaatt ataagaaatt ggtttcgttt ctttggaatt agggtttata 1800

```

```

ttactgttaa gattcgatta tagagtgaat tttgggaaga tttttcagat ttgatttgtg 1860
atgtgttgtg ttgtgagaaa ttgcagagtg tagtttcggg attaaacaga ggacttgtgg 1920
cgagtgttga tgatttagaa agagctgaag tggctgctaa agaacttgaa actgctgggg 1980
gaccggttga ttttaaccgat gatcttgata agcttcaagg gaaatggagg ctgttgtata 2040
gtagtgcggt ctcttctcgg tcttttaggtg gtagccgtcc tggctctacct actggacgtt 2100
tgatccctgt tactcttggc caggtaattc ttgaatcatt gctctgtttt taccctgcaa 2160
gattcggttt ttcgggtaag ttgttgagga gtttatgtgc atggtctagt ctatcatcaa 2220
tagtcttgct tgagtttgaa tggggctgag ctaagaatct agctttctga ggttacaatt 2280
tggtaatgtc atcttatact cgaaagcaaa cttttttcta tattgtcaag tttatgtgta 2340
cggctctggtc tatcattggt agtctttgtt gagcttgaat ggtgaggagc ttagaatcta 2400
gcaatgtcat ctactcctta atcatttttt tctatattgc caagtttatg tgtacgggtc 2460
tagtcaatca tctttattct tggttgagtt tgaatggtga tgagcttaga atctagcttt 2520
ctttggttta aatttggaag agaaccatac ctgaatcggg agaaagcaaa cttctttaat 2580
attatctctt gtttctgaat cattaaaaca ggtgtttcaa cggattgatg tgttagcaa 2640
agattttgat aacatagcag aggtggaatt aggagccctc tggccatttc cgccattaga 2700
agccactgcg acattggcac acaagtttga actcttaggt ttgcatttcc ctttctctca 2760
ttaagtttat cgaatttgtt aagagcaaaa taacttatct gtatctttga catttatggg 2820
gaaaacaggc acttgcaaga tcaagataac atttgagaaa acaactgtga agacatcggg 2880
aaacttgtcg cagattcctc cgtttgatat cccgaggctt cccgacagtt tcagaccatc 2940
gtcaaaccct ggaactgggg atttcgaaat tacctatgtt gatgatacca tgcgcataac 3000
tcgcggggac agaggtgaac ttagggtatt cgtcattgct taattctcaa agctttgaca 3060
tgtaaagata aataaatact ttctgcttga tgcagtctca tgagttttgt acaaatcatg 3120
tgaacatata aatgcgcttt ataagtaaag gagtgtcttg ttcaatgaat catatgaaag 3180
aatttgtatg actcagaaaa ttggacaatg atatagacct tccaaatttt gcacctcta 3240
atgtgagata ttagtgattt tttcttaggt tggtagagag aacggattgg caaaaaata 3300
tcgaaggtca atgattaaca gcaaaaccat atcttgatga ttcaaaatat agagttaaca 3360
agcaaagatg agacaatctt atacgagaga gctaaaacaa atggattcca aatccagcaa 3420
gtacaaaaat cgcagaaaat aagatgaaac caacttaaaa cagagatgtt ccctttccct 3480
tcttgtcacc accgatctcg aaatgcttgc acctctgaaa taaacaacaa accaacacaa 3540
tgtaagcaaa ttaccaagtt acaaaccggg tataatgaac tgatctatgt tctatgcacc 3600
ttgataggac gctgcgaaaa gtgcttgag ctttgacact gaagcctcaa aacaatcttc 3660
ttcgtggtct tagcctgtta acaagattca caagatgtat ctcagtccaa aactgagact 3720
attggaatgt ctgtttcctc acagctcact tccaaaattc tactataaat ggttccttaa 3780
aactacctca tttcaactaa ctagacctaa ttcaaaactga aaaaacaatc aatgcatgat 3840
aatcaatgtt acctttttgt ggaagacagg cttagtctga ccaccataac cagattgttt 3900
acggtcataa cgacgctttc cttgagcagc aagactgtct ttaccttct tgtattgggt 3960
aaccttgtgc aaagtatgct ttttgcattc cttgttctta cagtaagtgt tctttgtctt 4020
tggaatgttc accttcaaaa ttcataaaat caaaaatgaa tcaactcacac acatacaaaa 4080
tcaagagact ttttaaggta atcaaaatc aaacatcatt tagattgaaa acttttatga 4140
tagatctgaa aaacaatata ataaatcaat caaccatgta ttgttgttct tcaaagtc 4200
cgaactttac aaattccaaa atcacatcga aagagaagaa acaatttacc attttcgcgt 4260

```

&lt;210&gt; 4

&lt;211&gt; 203

&lt;212&gt; PRT

&lt;213&gt; oryza

&lt;400&gt; 4

Val Ala Ala Leu Lys Val Lys Leu Leu Ser Ala Val Ser Gly Leu Asn  
 1 5 10 15

Arg Gly Leu Ala Gly Ser Gln Glu Asp Leu Asp Arg Ala Asp Ala Ala  
 20 25 30

Ala Arg Glu Leu Glu Ala Ala Ala Gly Gly Gly Pro Val Asp Leu Glu  
 35 40 45

Arg Asp Val Asp Lys Leu Gln Gly Arg Trp Arg Leu Val Tyr Ser Ser  
 50 55 60

Ala Phe Ser Ser Arg Thr Leu Gly Gly Ser Arg Pro Gly Pro Pro Thr  
 65 70 75 80

Gly Arg Leu Leu Pro Ile Thr Leu Gly Gln Val Phe Gln Arg Ile Asp  
 85 90 95

Val Val Ser Lys Asp Phe Asp Asn Ile Val Asp Val Glu Leu Gly Ala  
 100 105 110

Pro Trp Pro Leu Pro Pro Val Glu Leu Thr Ala Thr Leu Ala His Lys  
 115 120 125

Phe Glu Ile Ile Gly Thr Ser Ser Ile Lys Ile Thr Phe Asp Lys Thr  
 130 135 140

Thr Val Lys Thr Lys Gly Asn Leu Ser Gln Leu Pro Pro Leu Glu Val  
 145 150 155 160

Pro Arg Ile Pro Asp Asn Leu Arg Pro Pro Ser Asn Thr Gly Ser Gly  
 165 170 175

Glu Phe Glu Val Thr Tyr Leu Asp Gly Asp Thr Arg Ile Thr Arg Gly  
 180 185 190

Asp Arg Gly Glu Leu Arg Val Phe Val Ile Ser  
 195 200

&lt;210&gt; 5

&lt;211&gt; 613

&lt;212&gt; DNA

&lt;213&gt; oryza

&lt;400&gt; 5

cgtggctgcg ctcaaagtca agcttctgag cgcggtgtcc gggctgaacc gcggcctcgc 60

ggggagccag gaggatcttg accgcgccga cgcggcggcg cgggagctcg aggcggcggc 120  
 ggggtggcggc cccgtcgacc tggagaggga cgtggacaag ctgcaggggc ggtggaggct 180  
 ggtgtacagc agcgcgttct cgtcgcggac gctcggcggc agccgccccg gcccgccac 240  
 cggccgcctc ctcccatca ccctcgggca ggtgtttcag aggatcgatg ttgtcagcaa 300  
 ggacttcgac aacatcgtcg atgtcgagct cggcgcgcca tggccgctgc cgccggtgga 360  
 gctgacggcg accctggctc acaagtttga gatcatcggc acctcgagca taaagatcac 420  
 attcgacaag acgacggtga agacgaaggg gaacctgtcc cagctgccgc cgctggaggt 480  
 ccctcgcatc ccggacaacc tccggccgcc gtccaacacc ggcagcggcg agttcgaggt 540  
 gacctacctc gacggcgaca cccgcatac cgcggggac agaggggagc tcagggtgtt 600  
 cgtcatctcg tga 613

&lt;210&gt; 6

&lt;211&gt; 26

&lt;212&gt; PRT

<213> *Xanthomonas campestris* pv. *glycines*

&lt;400&gt; 6

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala  
 1 5 10 15

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr  
 20 25

&lt;210&gt; 7

&lt;211&gt; 114

&lt;212&gt; PRT

<213> *Xanthomonas campestris* pv. *pelargonii*

&lt;400&gt; 7

Met Asp Ser Ile Gly Asn Asn Phe Ser Asn Ile Gly Asn Leu Gln Thr  
 1 5 10 15

Met Gly Ile Gly Pro Gln Gln His Glu Asp Ser Ser Gln Gln Ser Pro  
 20 25 30

Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln Leu Leu Ala Met Phe Ile  
 35 40 45

Met Met Met Leu Gln Gln Ser Gln Gly Ser Asp Ala Asn Gln Glu Cys  
 50 55 60

Gly Asn Glu Gln Pro Gln Asn Gly Gln Gln Glu Gly Leu Ser Pro Leu  
 65 70 75 80

Thr Gln Met Leu Met Gln Ile Val Met Gln Leu Met Gln Asn Gln Gly  
 85 90 95

Gly Ala Gly Met Gly Gly Gly Gly Ser Val Asn Ser Ser Leu Gly Gly  
 100 105 110

Asn Ala

<210> 8

<211> 342

<212> DNA

<213> *Xanthomonas campestris* pv. *pelargonii*

<400> 8

```
atggactcta tcggaacaa cttttcgaat atcggcaacc tgcagacgat gggcatcggg 60
cctcagcaac acgaggactc cagccagcag tcgccttcgg ctggctccga gcagcagctg 120
gatcagttgc tcgccatggt catcatgatg atgctgcaac agagccaggg cagcagtgca 180
aatcaggagt gtggcaacga acaaccgcag aacgggtcaac aggaaggcct gagtccgttg 240
acgcagatgc tgatgcagat cgtgatgcag ctgatgcaga accagggcgg cgccggcatg 300
ggcgggtggcg gttcgggtcaa cagcagcctg ggcggcaacg cc 342
```

<210> 9

<211> 205

<212> DNA

<213> Artificial Sequence.

<220>

<223> Description of Artificial Sequence: probe

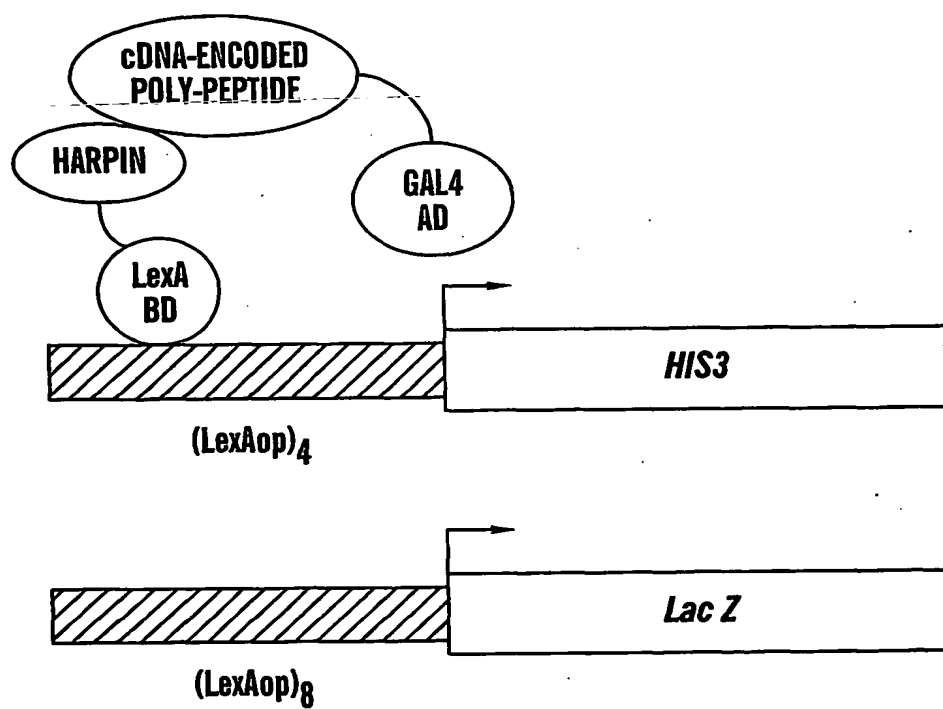
<400> 9

```
gatcaagata acatttgaga aaacaactgt gaagacatcg ggaaacttgt cgcagattcc 60
tcggtttgat atcccagggc ttcccgacag ttccagacca tcgtcaaacc ctggaactgg 120
ggatttcgaa gttacctatg ttgatgatac catgcgcata actcgcgggg acagaggtga 180
acttagggta ttcgtcattg cttaa 205
```

**THIS PAGE BLANK (USPTO)**

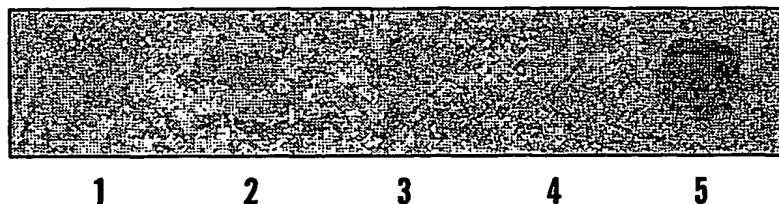
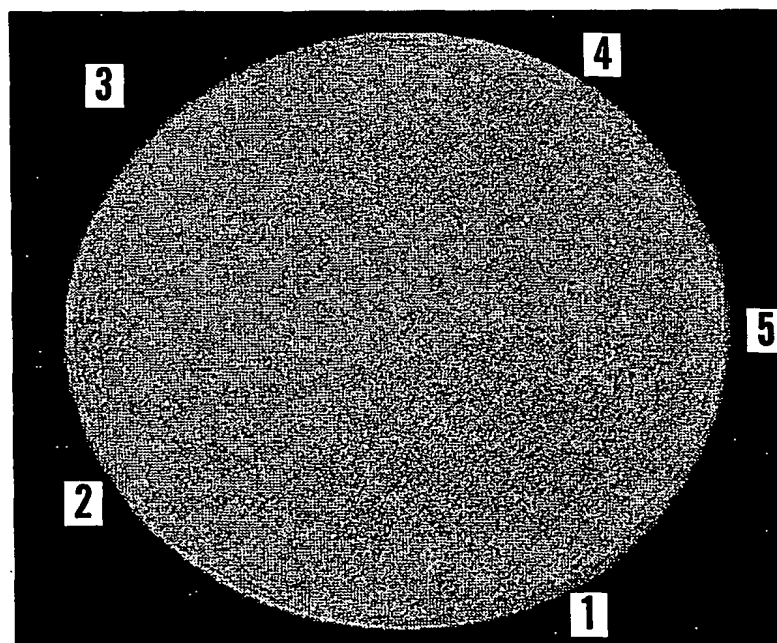


1/12

**FIG. 1**

**THIS PAGE BLANK (USPTO)**

2/12

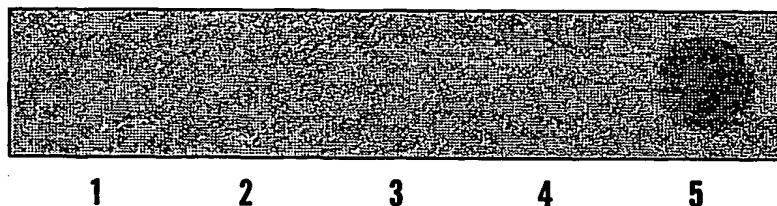
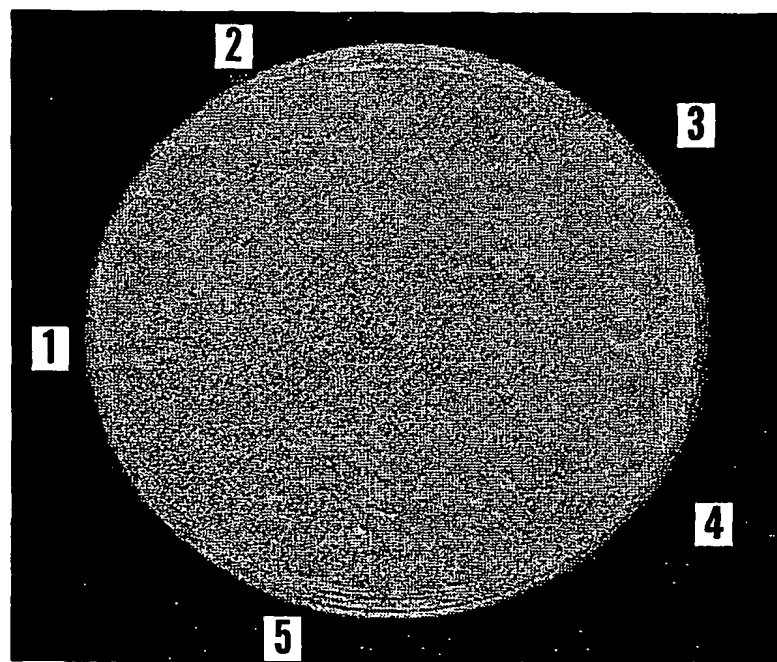
**FIG. 2A**

	BAIT	PREY
1	HARPIN	EMPTY VECTOR
2	HARPIN	UNRELATED PROTEIN
3	EMPTY VECTOR	HARPIN
4	UNRELATED PROTEIN	HARPIN
5	(POSITIVE CONTROL)	

**FIG. 2B**

**THIS PAGE BLANK (USPTO)**

3/12

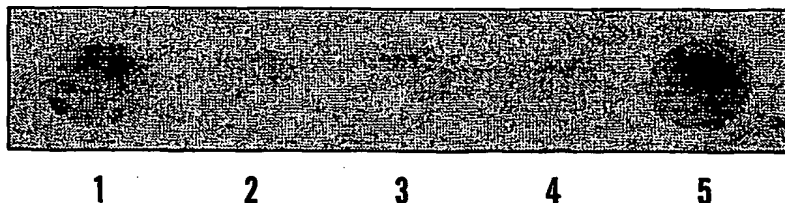
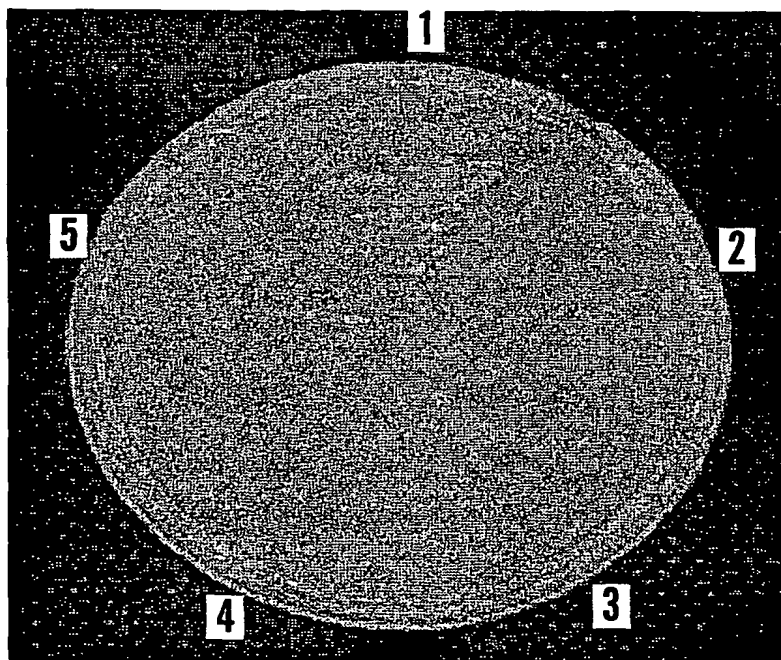
**FIG. 3A**

	BAIT	PREY
1	HARPIN	HrBP1
2	UNRELATED PROTEIN 1	HrBP1
3	UNRELATED PROTEIN 2	HrBP1
4	EMPTY VECTOR	HrBP1
5	(POSITIVE CONTROL)	

**FIG. 3B**

**THIS PAGE BLANK (USPTO)**

4/12

**FIG. 4A**

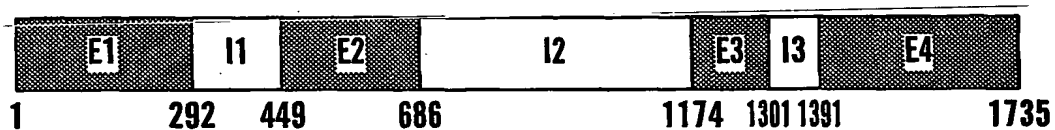
	BAIT	PREY
1	HrBP1	HARPIN
2	HrBP1	EMPTY VECTOR
3	HrBP1	UNRELATED PROTEIN 1
4	HrBP1	UNRELATED PROTEIN 2
5	(POSITIVE CONTROL)	

**FIG 4B**

**THIS PAGE BLANK (USPTO)**



5/12



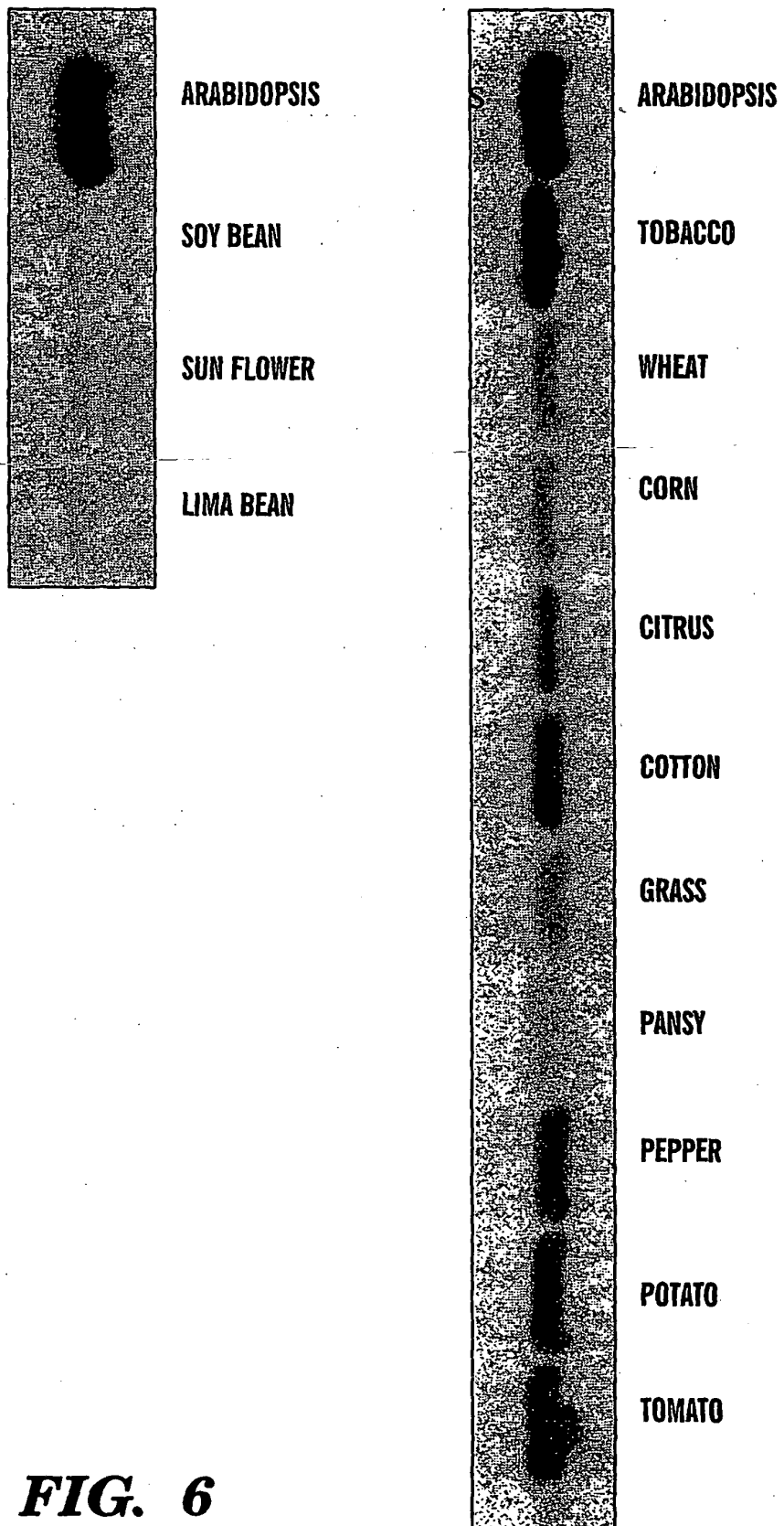
GENE STRUCTURE OF HrBP1

E: EXON

I: INTRON

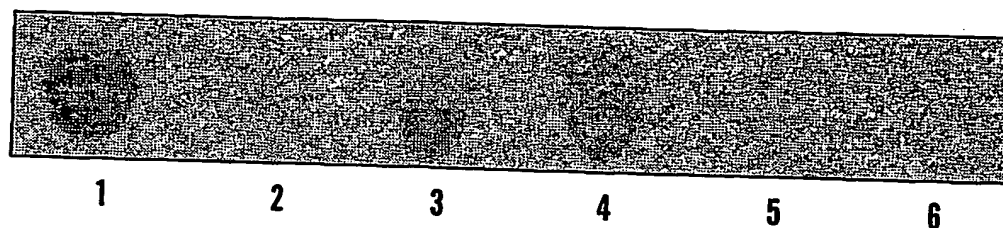
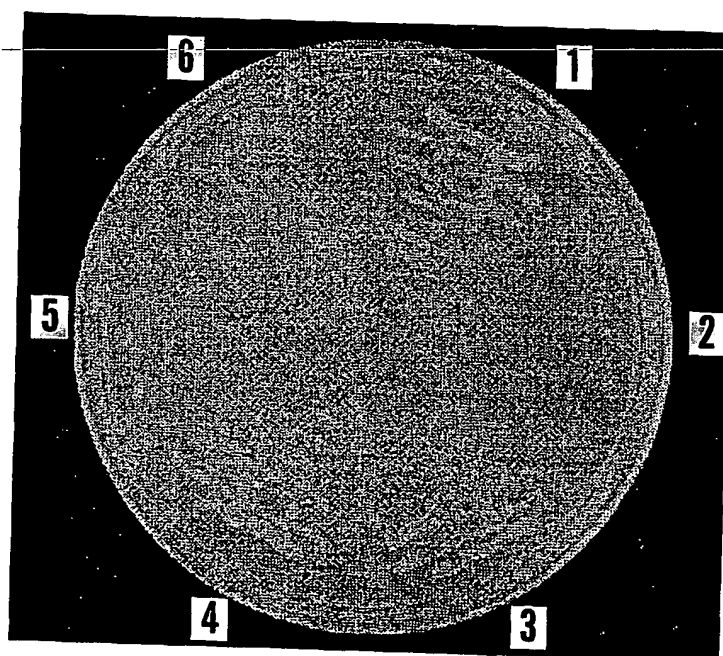
**FIG. 5**

**THIS PAGE BLANK (USPTO)**



**FIG. 6**

**THIS PAGE BLANK (USPTO)**

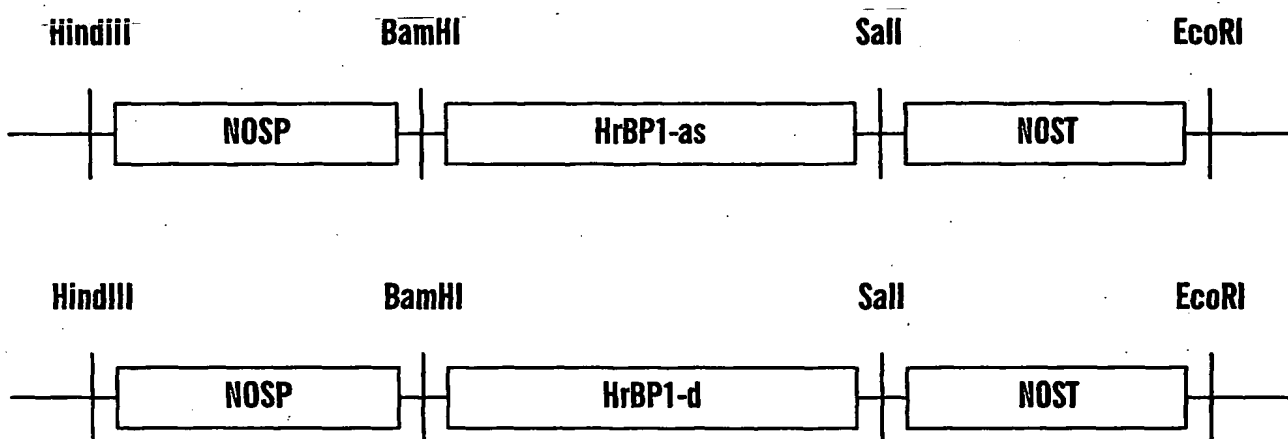
**FIG. 7A**

	BAIT	PREY
1	POSITIVE CONTROL	
2	pVJL11	R6
3	hrpN FULL LENGTH	R6
4	hrpN 137-180aa	R6
5	hrpN 210-403aa	R6
6	lexA-lamin	R6

**FIG. 7B**

**THIS PAGE BLANK (USPTO)**

8/12



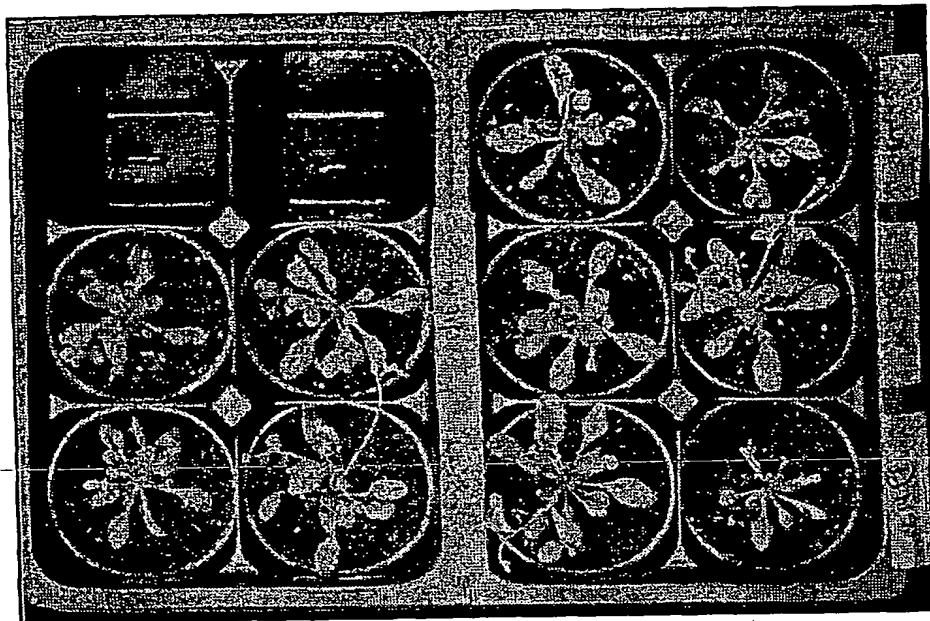
**HrBP1-d: HrBP1 CODING REGION BASES 4-650 SENSE STRAND + 4-500 ANTI-SENSE STRAND**

**FIG. 8**

**THIS PAGE BLANK (USPTO)**



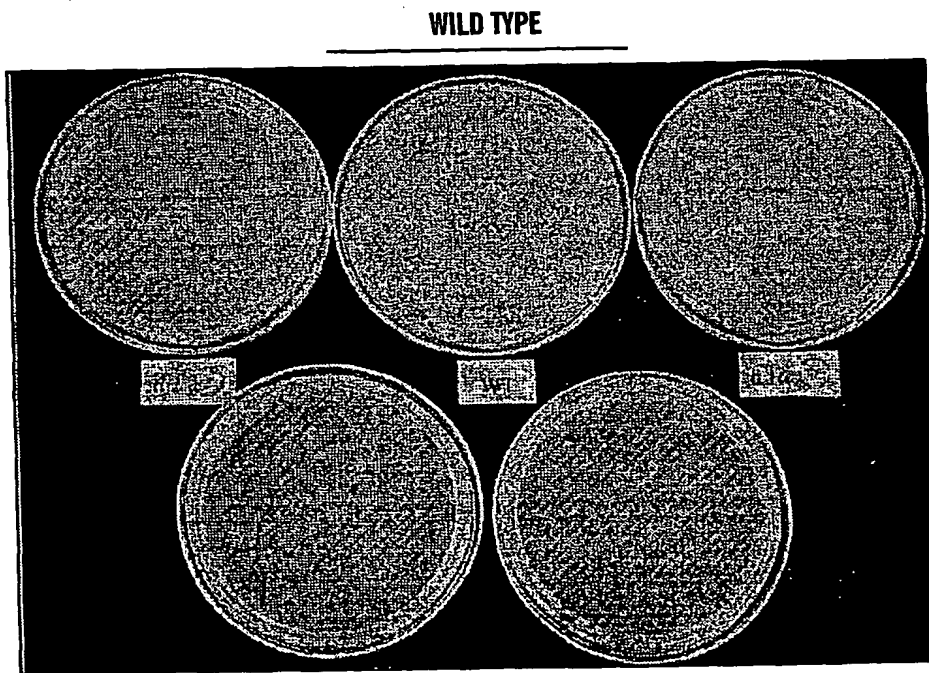
9/12



WILD TYPE

TRANSGENIC LINES

**FIG. 9A**



WILD TYPE

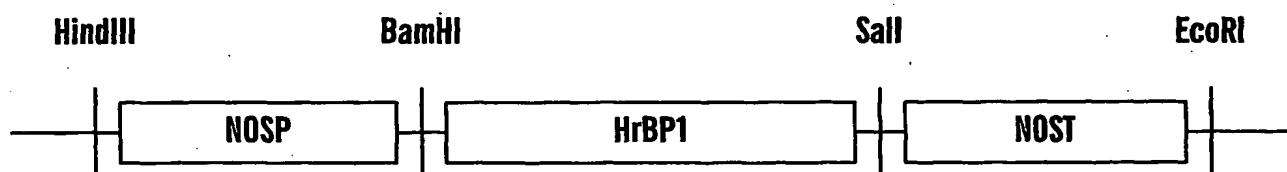
**FIG. 9B**

	cfu/cm <sup>2</sup>
WILD TYPE	$5.5 \times 10^7$
d2-2	$2.0 \times 10^5$
d16-9	$2.0 \times 10^5$
as14-7	$2.1 \times 10^6$
as17-8	$4.1 \times 10^6$

**FIG 9C**

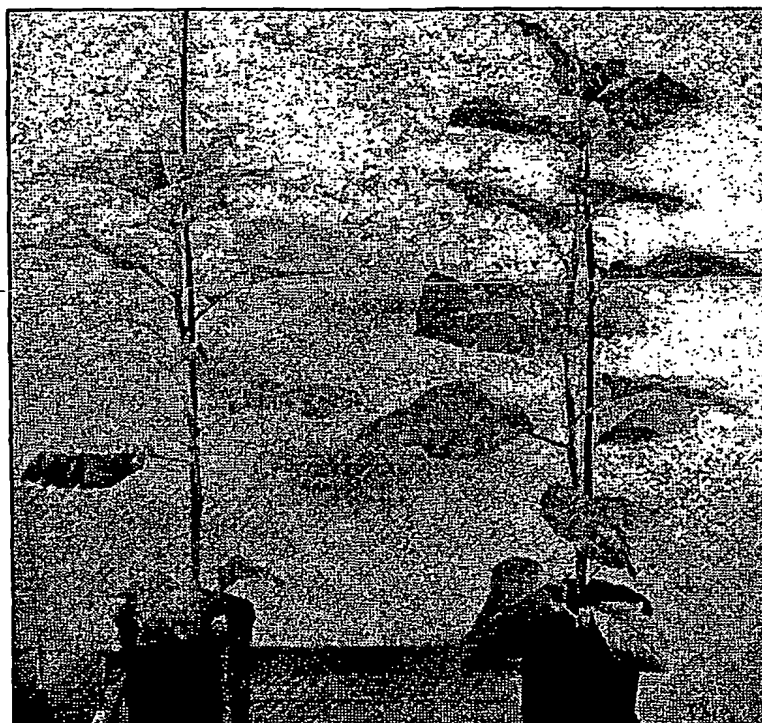
**THIS PAGE BLANK (USPTO)**

10/12

**FIG. 10**

**THIS PAGE BLANK (USPTO)**

11/12



WILD TYPE

HrPP1 OVER-EXPRESSING  
TOBACCO**FIG. 11A**

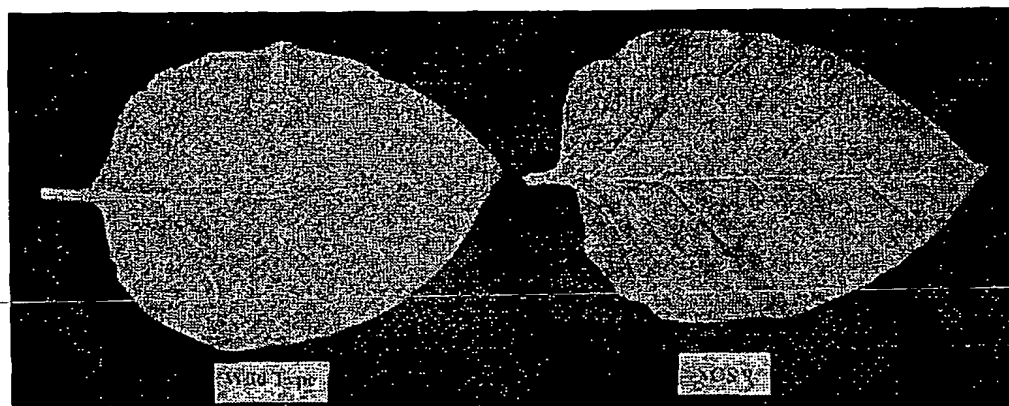
HEIGHT OF T2 TOBACCO PLANTS AFTER 52 DAYS TRANSFERRING TO SOIL

WILD TYPE	$49.5 \pm 3.2$ cm
LINE 1	$59.5 \pm 3.8$ cm
LINE 2	$61.7 \pm 1.9$ cm
LINE 3	$64.3 \pm 5.5$ cm

**FIG. 11B**

**THIS PAGE BLANK (USPTO)**

12/12



WILD TYPE

HrBP1 OVER-EXPRESSING LINE

**FIG. 12A**

	DIAMETER OF DISEASE SPOT
WILD TYPE	$2.97 \pm 0.49\text{mm}$
LINE 1	$1.97 \pm 0.31\text{mm}$
LINE 2	$1.95 \pm 0.37\text{mm}$
LINE 3	$2.01 \pm 0.33\text{mm}$

**FIG. 12B**

**THIS PAGE BLANK (USPTO)**





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C07K 14/195, C12N 15/31, 1/21, 5/10,</b> <b>A01H 5/00, 5/10, C12N 15/82</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 00/20452</b> <b>(43) International Publication Date:</b> 13 April 2000 (13.04.00)
<b>(21) International Application Number:</b> PCT/US99/23181 <b>(22) International Filing Date:</b> 5 October 1999 (05.10.99)  <b>(30) Priority Data:</b> 60/103,050 5 October 1998 (05.10.98) US  <b>(71) Applicant:</b> EDEN BIOSCIENCE CORPORATION [US/US]; 11816 North Creek Parkway N., Bothell, WA 98011-8205 (US).  <b>(72) Inventors:</b> WEI, Zhong-Min; 8230 125th Court, Kirkland, WA 98034 (US). FAN, Hao; 19712 6th Drive S.E., Bothell, WA 98012 (US). NIGGEMEYER, Jennifer, L.; 21315 2nd Avenue S.E., Bothell, WA 98021 (US).  <b>(74) Agents:</b> GOLDMAN, Michael, L. et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>  <b>(88) Date of publication of the international search report:</b> 6 July 2000 (06.07.00)
<b>(54) Title:</b> HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSEN- SITIVE RESPONSE  <b>(57) Abstract</b>  The present invention is directed to isolated active fragments of a hypersensitive response elicitor protein or polypeptide which fragment does not elicit a hypersensitive response in plants. Also disclosed are isolated DNA molecules which encode such fragments. Isolated fragments of hypersensitive response elicitor proteins or polypeptides in accordance with the present invention and the isolated DNA molecules that encode them have the following activities: imparting disease resistance to plants, enhancing plant growth, and/or controlling insects on plants. This can be achieved by applying the fragments of a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the fragment can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakistan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

SEP 28 2000

## **HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE**

This application claims benefit of U.S. Provisional Patent Application  
5 Serial No. 60/103,050, filed October 5, 1998.

### **FIELD OF THE INVENTION**

The present invention relates to active fragments of a hypersensitive  
10 response elicitor which fragments do not elicit a hypersensitive response.

### **BACKGROUND OF THE INVENTION**

Interactions between bacterial pathogens and their plant hosts generally  
15 fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations  
20 increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.

The hypersensitive response is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z.,  
25 "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily  
30 observed as a tissue collapse if high concentrations ( $\geq 10^7$  cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

- 2 -

“Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf,” Phytopathology 54:474-477 (1963); Turner, et al., “The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction,” Phytopathology 64:885-890 (1974); Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., “Gene Cluster of *Pseudomonas syringae* pv. ‘phaseolicola’ Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants,” J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., “*hrp* Genes of Phytopathogenic Bacteria,” pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., “Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria,” Trends Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich protein elicitors of the hypersensitive response (He, S.Y., et al. “*Pseudomonas Syringae* pv. *Syringae* HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants,” Cell 73:1255-1266 (1993), Wei, Z.-H.,

- 3 -

et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "Erwinia chrysanthemi Harpin<sub>Ech</sub>: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA<sup>-</sup> Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention seeks to identify fragments of hypersensitive response elicitor proteins or polypeptides, which fragments do not elicit a hypersensitive response but are active when utilized in conjunction with plants.

## SUMMARY OF THE INVENTION

5

The present invention is directed to isolated fragments of an *Erwinia* hypersensitive response elicitor protein or polypeptide which fragments do not elicit a hypersensitive response in plants but are otherwise active when utilized in conjunction with plants. Also disclosed are isolated DNA molecules which encode such fragments.

10

The fragments of hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth and/or controlling insects. This involves applying the fragments in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

15

As an alternative to applying the fragments to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a fragment can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

20

25

30

- 5 -

### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows truncated proteins of the hypersensitive response elicitor protein or polypeptide.

5           Figure 2 shows a list of synthesized oligonucleotide primers for construction of truncated harpin proteins. N represents the N-terminus (5' region), and C represents the C-terminus (3' region). The primers correspond to the indicated sequence identification numbers for the present application: N1 (SEQ. ID. No. 1), N76 (SEQ. ID. No. 2), N99 (SEQ. ID. No. 3), N105 (SEQ. ID. No. 4), N110 (SEQ. ID. No. 5), N137 (SEQ. ID. No. 6), N150 (SEQ. ID. No. 7), N169 (SEQ. ID. No. 8),  
10   N210 (SEQ. ID. No. 9), N267 (SEQ. ID. No. 10), N343 (SEQ. ID. No. 11), C75 (SEQ. ID. No. 12), C104 (SEQ. ID. No. 13), C168 (SEQ. ID. No. 14), C180 (SEQ. ID. No. 15), C204 (SEQ. ID. No. 16), C209 (SEQ. ID. No. 17), C266 (SEQ. ID. No. 18), C342 (SEQ. ID. No. 19), and C403 (SEQ. ID. No. 20).

15

### **DETAILED DESCRIPTION OF THE INVENTION**

The present invention is directed to isolated fragments of a hypersensitive response elicitor protein or polypeptide where the fragments do not  
20   elicit a hypersensitive response but have other activity in plants. Also disclosed are DNA molecules encoding such fragments as well as expression systems, host cells, and plants containing such molecules. Uses of the fragments themselves and the DNA molecules encoding them are disclosed.

The fragments of hypersensitive response elicitor polypeptides or  
25   proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein  
30   elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

- 6 -

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

5 The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 21 as follows:

10	Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser	1	5	10	15
	Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser	20	25	30	
	Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr	35	40	45	
15	Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu	50	55	60	
	Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser	65	70	75	80
20	Phe	Gly	Asn	Gly	Ala	Gln	Gly	Ala	Ser	Asn	Leu	Leu	Ser	Val	Pro	Lys	85	90	95	
	Ser	Gly	Gly	Asp	Ala	Leu	Ser	Lys	Met	Phe	Asp	Lys	Ala	Leu	Asp	Asp	100	105	110	
	Leu	Leu	Gly	His	Asp	Thr	Val	Thr	Lys	Leu	Thr	Asn	Gln	Ser	Asn	Gln	115	120	125	
25	Leu	Ala	Asn	Ser	Met	Leu	Asn	Ala	Ser	Gln	Met	Thr	Gln	Gly	Asn	Met	130	135	140	
	Asn	Ala	Phe	Gly	Ser	Gly	Val	Asn	Asn	Ala	Leu	Ser	Ser	Ile	Leu	Gly	145	150	155	160
30	Asn	Gly	Leu	Gly	Gln	Ser	Met	Ser	Gly	Phe	Ser	Gln	Pro	Ser	Leu	Gly	165	170	175	
	Ala	Gly	Gly	Leu	Gln	Gly	Leu	Ser	Gly	Ala	Gly	Ala	Phe	Asn	Gln	Leu	180	185	190	
	Gly	Asn	Ala	Ile	Gly	Met	Gly	Val	Gly	Gln	Asn	Ala	Ala	Leu	Ser	Ala	195	200	205	
35	Leu	Ser	Asn	Val	Ser	Thr	His	Val	Asp	Gly	Asn	Asn	Arg	His	Phe	Val	210	215	220	
	Asp	Lys	Glu	Asp	Arg	Gly	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met	Asp	225	230	235	240



- 7 -

[illegible]

15 This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence  
20 corresponding to SEQ. ID. No. 22 as follows:

	CGATTTTACC	CGGGTGAACG	TGCTATGACC	GACAGCATCA	CGGTATTCCA	CACCGTTACG	60
	GCGTTTATGG	CCGCGATGAA	CCGGCATCAG	GCGGCGCGCT	GGTCGCCGCA	ATCCGGCGTC	120
	GATCTGGTAT	TTCAGTTTGG	GGACACCGGG	CGTGAACCTCA	TGATGCAGAT	TCAGCCGGGG	180
25	CAGCAATATC	CCGGCATGTT	GCGCACGCTG	CTCGCTCGTC	GTTATCAGCA	GGCGGCAGAG	240
	TGCGATGGCT	GCCATCTGTG	CCTGAACGGC	AGCGATGTAT	TGATCCTCTG	GTGGCCGCTG	300
	CCGTCGGATC	CCGGCAGTTA	TCCGCAGGTG	ATCGAACGTT	TGTTTGAAC	GGCGGGAATG	360
	ACGTTGCCGT	CGCTATCCAT	AGCACCGACG	GCGCGTCCGC	AGACAGGGAA	CGGACGCGCC	420
	CGATCATTAA	GATAAAGGCG	GCTTTTTTTT	TTGCAAAACG	GTAACGGTGA	GGAACCGTTT	480
30	CACCGTCGGC	GTCACTCAGT	AACAAGTATC	CATCATGATG	CCTACATCGG	GATCGGCGTG	540
	GGCATCCGTT	GCAGATACTT	TTGCGAACAC	CTGACATGAA	TGAGGAAACG	AAATTATGCA	600
	AATTACGATC	AAAGCGCACA	TCGGCGGTGA	TTTGGGCGTC	TCCGGTCTGG	GGCTGGGTGC	660
	TCAGGGACTG	AAAGGACTGA	ATTCCGCGGC	TTCATCGCTG	GGTTCAGCG	TGGATAAACT	720
	GAGCAGCACC	ATCGATAAGT	TGACCTCCGC	GCTGACTTCG	ATGATGTTTG	GCGGCGCGCT	780
35	GGCGCAGGGG	CTGGGCGCCA	GCTCGAAGGG	GCTGGGGATG	AGCAATCAAC	TGGGCCAGTC	840

- 8 -

TTTCCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA 900  
 TGC GTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC 960  
 CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC 1020  
 CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG 1080  
 5 CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT 1140  
 GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT 1200  
 GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA 1260  
 CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA 1320  
 TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA 1380  
 10 GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG 1440  
 CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA 1500  
 TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC 1560  
 GGCTGTGCTC GGCATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA 1620  
 ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC 1680  
 15 TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA 1740  
 ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC 1800  
 GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC 1860  
 CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCTTTAG 1920  
 CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG 1980  
 20 GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC 2040  
 AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG 2100  
 GTTCGTCATC ATCTTCTCC ATCTGGGCGA CCTGATCGGT T 2141

25 The hypersensitive response elicitor polypeptide or protein derived  
 from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID.  
 No. 23 as follows:

30 Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
 1 5 10 15  
 Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
 20 25 30

- 9 -

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
 35 40 45  
 Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
 50 55 60  
 5 Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
 65 70 75 80  
 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
 85 90 95  
 10 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
 100 105 110  
 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
 115 120 125  
 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
 130 135 140  
 15 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
 145 150 155 160  
 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
 165 170 175  
 20 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu  
 180 185 190  
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly  
 195 200 205  
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly  
 210 215 220  
 25 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu  
 225 230 235 240  
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln  
 245 250 255  
 30 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln  
 260 265 270  
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe  
 275 280 285  
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met  
 290 295 300  
 35 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro  
 305 310 315 320  
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser  
 325 330 335  
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn

This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 24 as follows:

20	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTATATA	60
	GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT	120
	ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
	GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG	240
	GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
25	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
	GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTTCG TGAACACGCT GGGCTCGAAA	420
	GGCGGCAACA ATACCACTTC AACACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
	TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
	CCGATGCAGC AGCTGCTGAA GATGTTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
30	CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
	GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
	CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
	GGTTGCTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG	840

- 11 -

TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTGAGG GCTGAATGAT 900  
 ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG 960  
 GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC 1020  
 CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC 1080  
 5 AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC 1140  
 ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC 1200  
 GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA 1260  
 CTTGGCAAGC TGGGCGCGGC TTAAGCTT 1288

10

Another potentially suitable hypersensitive response elicitor from  
*Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927,  
 which is hereby incorporated by reference. The protein is encoded by a DNA  
 molecule having a nucleic acid sequence of SEQ. ID. No. 25 as follows:

15 ATGTCAATTC TTACGCTTAA CAACAATACC TCGTCCTCGC CGGTCTGTT CCAGTCCGGG 60  
 GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT 120  
 20 CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGGAAC TGTTAAAGTC ACTGCTATCG 180  
 CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT 240  
 AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT 300  
 25 CAGAACATGC TGAGTGAGAT GGGCAACAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC 360  
 CAGGGCGGCG GGCAGATCGG CGATAATCCT TTAGTGAAAG CCATGCTGAA GCTTATTGCA 420  
 30 CGCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC 480  
 TCTTCCGGTA CTCTTCATC TGGCGGTTCC CCTTTAAACG ATCTATCAGG GGGGAAGGCC 540  
 CCTTCCGGCA ACTCCCCTTC CGGCAACTAC TCTCCGTC GTACCTTCTC ACCCCCATCC 600  
 35 ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCCTCTT CTCCCACCA AGCAGCCGGG 660  
 GGCAGCACGC CGGTAACCGA TCATCCTGAC CTTGTTGGTA GCGCGGGCAT CGGGGCCGGA 720  
 40 AATTCGGTGG CCTTCACCAG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC 780  
 GTGAAAGCGG GTCAGGTGTT TGATGGCAAA GGACAAACCT TCACCGCCGG TTCAGAATTA 840  
 GGCATGCGC GCCAGTCTGA AAACCAGAAA CCGCTGTTTA TACTGGAAGA CGGTGCCAGC 900  
 45 CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC 960  
 AAAATAGACA ATCTGCAGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC 1020  
 50 AGCGCGGGCA AAAAATCCCA CGTTGAAATC ACTAACAGTT CCTTCGAGCA CGCCTCTGAC 1080  
 AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC 1140

- 12 -

TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC 1200  
 CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC 1260  
 5 GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC 1320  
 GCCAACCTGA AGGTGGCTGA ATGA 1344

10

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 26 as follows:

15 Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu  
 1 5 10 15  
 Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser  
 20 20 25 30  
 Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala  
 35 40 45  
 Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly  
 25 50 55 60  
 Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly  
 65 70 75 80  
 30 Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro  
 85 90 95  
 Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu  
 100 105 110  
 35 Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp  
 115 120 125  
 Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp  
 40 130 135 140  
 Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala  
 145 150 155 160  
 45 Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser  
 165 170 175  
 Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro  
 180 185 190  
 50 Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro  
 195 200 205  
 Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro  
 55 210 215 220

- 13 -

Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly  
 225 230 235 240  
 5 Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His  
 245 250 255  
 Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln  
 260 265 270  
 10 Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn  
 275 280 285  
 Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val  
 290 295 300  
 15 Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala  
 305 310 315 320  
 20 Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr  
 325 330 335  
 Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn  
 340 345 350  
 25 Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp  
 355 360 365  
 Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe  
 370 375 380  
 30 Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser  
 385 390 395 400  
 His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser  
 405 410 415  
 35 Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu  
 420 425 430  
 40 Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu  
 435 440 445

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It  
 45 is also heat stable, protease sensitive, and suppressed by inhibitors of plant  
 metabolism. The protein or polypeptide of the present invention has a predicted  
 molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from  
*Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663  
 50 which is hereby incorporated by reference. The protein is encoded by a DNA  
 molecule having a nucleic acid sequence of SEQ. ID. No. 27 as follows:

- 14 -

	ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAC	60
	CCTGTGGGGC ATGGTGTTC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC	120
5	GCTGCATCAT TGGCGGCAGA AGGCAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA	180
	TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG	240
	GGCTGTTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCACC	300
10	CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT	360
	GAGGCGGCCG CGCCAGATGC GGCGCGTTTG ACCCGTTCGG GCGGCGTCAA ACGCCGCAAT	420
15	ATGGACGACA TGGCCGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA	480
	ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC	540
	AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGCGATCGCC TGCAGCATTC ACCGCCGCAC	600
20	ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA	660
	ACGGCCACAG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA	720
25	CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC	780
	GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAACTGA CTGCGGTTGC GGAAAGCGTC	840
	CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT	900
30	GGAGCCGGGG TAACGCCGCT GGCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG	960
	GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC	1020
35	TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC	1080
	CACCTGTTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC	1140
	GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA	1200
40	AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAACAAT GCTAAGCCAA	1260
	CCGGGGGAAG CGCACCCTTC CTTATTAACC GGCAATTGGC AGCATCCTGC TGGCGCAGCG	1320
45	CGGCCGCAGG GCGAGTCAAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG	1380
	CTGGGCGTAT GGCAATCTGC GGATAAAGAT ACCCACAGCC AGCTGTCTCG CCAGGCAGAC	1440
	GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCCGA TAATAAATCC	1500
50	TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG	1560
	ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC	1620
55	CCGGAGAGCC ATATTTCCCT CAGCCTGCAT TTTGCCGATG CCCACCAGGG GTTATTGCAC	1680
	GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTGTGGCC	1740
	GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG	1800
60	AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT	1860
	TCTGGATTTT TCCATGACGA CCACGGCCAG CTTAATGCGC TGGTGAAAAA TAACTTCAGG	1920
65	CAGCAGCATG CCTGCCCGTT GGGTAACGAT CATCAGTTTC ACCCGGCTG GAACCTGACT	1980



- 15 -

	GATGCGCTGG	TTATCGACAA	TCAGCTGGGG	CTGCATCATA	CCAATCCTGA	ACCGCATGAG	2040
	ATTCTTGATA	TGGGGCATT	AGGCAGCCTG	GCGTTACAGG	AGGGCAAGCT	TCACTATTTT	2100
5	GACCAGCTGA	CCAAAGGGTG	GA CTGGCGCG	GAGTCAGATT	GTAAGCAGCT	GAAAAAAGGC	2160
	CTGGATGGAG	CAGCTTATCT	ACTGAAAGAC	GGTGAAGTGA	AACGCCTGAA	TATTAATCAG	2220
10	AGCACCTCCT	CTATCAAGCA	CGGAACGGAA	AACGTTTTTT	CGCTGCCGCA	TGTGCGCAAT	2280
	AAACCGGAGC	CGGGAGATGC	CCTGCAAGGG	CTGAATAAAG	ACGATAAGGC	CCAGGCCATG	2340
	GCGGTGATTG	GGGTAAATAA	ATACCTGGCG	CTGACGGAAA	AAGGGGACAT	TCGCTCCTTC	2400
15	CAGATAAAAC	CCGGCACCCA	GCAGTTGGAG	CGGCCGGCAC	AAACTCTCAG	CCGCGAAGGT	2460
	ATCAGCGGCG	AACTGAAAGA	CATTTCATGTC	GACCACAAGC	AGAACCTGTA	TGCCTTGACC	2520
20	CACGAGGGAG	AGGTGTTTCA	TCAGCCGCGT	GAAGCCTGGC	AGAATGGTGC	CGAAAGCAGC	2580
	AGCTGGCACA	AACTGGCGTT	GCCACAGAGT	GAAAGTAAGC	TAAAAAGTCT	GGACATGAGC	2640
	CATGAGCACA	AACCGATTGC	CACCTTTGAA	GACGGTAGCC	AGCATCAGCT	GAAGGCTGGC	2700
25	GGCTGGCACG	CCTATGCGGC	ACCTGAACGC	GGGCCGCTGG	CGGTGGGTAC	CAGCGGTTCA	2760
	CAAACCGTCT	TTAACCGACT	AATGCAGGGG	GTGAAAGGCA	AGGTGATCCC	AGGCAGCGGG	2820
30	TTGACGGTTA	AGCTCTCGGC	TCAGACGGGG	GGAATGACCG	GCGCCGAAGG	GCGCAAGGTC	2880
	AGCAGTAAAT	TTTCCGAAAG	GATCCGCGCC	TATGCGTTCA	ACCCAACAAT	GTCCACGCCG	2940
	CGACCGATTA	AAAATGCTGC	TTATGCCACA	CAGCACGGCT	GGCAGGGGCG	TGAGGGGTTG	3000
35	AAGCCGTTGT	ACGAGATGCA	GGGAGCGCTG	ATTAAACAAC	TGGATGCGCA	TAACGTTCTG	3060
	CATAACGCGC	CACAGCCAGA	TTTGCAGAGC	AAACTGGAAA	CTCTGGATTT	AGGCGAACAT	3120
40	GGCGCAGAAT	TGCTTAACGA	CATGAAGCGC	TTCCGCGACG	AACTGGAGCA	GAGTGCAACC	3180
	CGTTCGGTGA	CCGTTTTAGG	TCAACATCAG	GGAGTGCTAA	AAAGCAACGG	TGAAATCAAT	3240
	AGCGAATTTA	AGCCATCGCC	CGGCAAGGCG	TTGGTCCAGA	GCTTTAACGT	CAATCGCTCT	3300
45	GGTCAGGATC	TAAGCAAGTC	ACTGCAACAG	GCAGTACATG	CCACGCCGCC	ATCCGCAGAG	3360
	AGTAAACTGC	AATCCATGCT	GGGGCACTTT	GTCAGTGCCG	GGGTGGATAT	GAGTCATCAG	3420
50	AAGGGCGAGA	TCCCGCTGGG	CCGCCAGCGC	GATCCGAATG	ATAAAACCGC	ACTGACCAAA	3480
	TCGCGTTTAA	TTTTAGATAC	CGTGACCATC	GGTGAAGTGC	ATGAACTGGC	CGATAAGGCG	3540
	AAACTGGTAT	CTGACCATAA	ACCCGATGCC	GATCAGATAA	AACAGCTGCG	CCAGCAGTTC	3600
55	GATACGCTGC	GTGAAAAGCG	GTATGAGAGC	AATCCGGTGA	AGCATTACAC	CGATATGGGC	3660
	TTCACCCATA	ATAAGGCGCT	GGAAGCAAAC	TATGATGCGG	TCAAAGCCTT	TATCAATGCC	3720
60	TTTAAGAAAG	AGCACCACGG	CGTCAATCTG	ACCACGCGTA	CCGTACTGGA	ATCACAGGGC	3780
	AGTGCGGAGC	TGGCGAAGAA	GCTCAAGAAT	ACGCTGTTGT	CCCTGGACAG	TGGTGAAAGT	3840
65	ATGAGCTTCA	GCCGGTCATA	TGGCGGGGGC	GTCAGCACTG	TCTTTGTGCC	TACCCCTTAGC	3900

- 16 -

	AAGAAGGTGC	CAGTTCCGGT	GATCCCCGGA	GCCGGCATCA	CGCTGGATCG	CGCCTATAAC	3960
	CTGAGCTTCA	GTCGTACCAG	CGGCGGATTG	AACGTCAGTT	TTGGCCGCGA	CGGCGGGGTG	4020
5	AGTGGTAACA	TCATGGTCGC	TACCGGCCAT	GATGTGATGC	CCTATATGAC	CGGTAAGAAA	4080
	ACCACTGCAG	GTAACGCCAG	TGACTGGTTG	AGCGCAAAAC	ATAAAATCAG	CCCGGACTTG	4140
10	CGTATCGGCG	CTGCTGTGAG	TGGCACCCGT	CAAGGAACGC	TACAAAACAG	CCTGAAGTTT	4200
	AAGCTGACAG	AGGATGAGCT	GCCTGGCTTT	ATCCATGGCT	TGACGCATGG	CACGTTGACC	4260
	CCGGCAGAAC	TGTTGCAAAA	GGGGATCGAA	CATCAGATGA	AGCAGGGCAG	CAAACGACG	4320
15	TTTAGCGTCG	ATACCTCGGC	AAATCTGGAT	CTGCGTGCCG	GTATCAATCT	GAACGAAGAC	4380
	GGCAGTAAAC	CAAATGGTGT	CACTGCCCCG	GTTTCTGCCG	GGCTAAGTGC	ATCGGCAAAC	4440
20	CTGGCCGCCG	GCTCGCGTGA	ACGCAGCACC	ACCTCTGGCC	AGTTTGGCAG	CACGACTTCG	4500
	GCCAGCAATA	ACCGCCCAAC	CTTCCTCAAC	GGGGTCGGCG	CGGGTGCTAA	CCTGACGGCT	4560
	GCTTTAGGGG	TTGCCCATTG	ATCTACGCAT	GAAGGGAAAC	CGGTCGGGAT	CTTCCCGGCA	4620
25	TTTACCTCGA	CCAATGTTTC	GGCAGCGCTG	GCGCTGGATA	ACCGTACCTC	ACAGAGTATC	4680
	AGCCTGGAAT	TGAAGCGCGC	GGAGCCGGTG	ACCAGCAACG	ATATCAGCGA	GTTGACCTCC	4740
30	ACGCTGGGAA	AACACTTTAA	GGATAGCGCC	ACAACGAAGA	TGCTTGCCGC	TCTCAAAGAG	4800
	TTAGATGACG	CTAAGCCCGC	TGAACAACTG	CATATTTTAC	AGCAGCATTT	CAGTGCAAAA	4860
	GATGTCGTCG	GTGATGAACG	CTACGAGGCG	GTGCGCAACC	TGAAAAAACT	GGTGATACGT	4920
35	CAACAGGCTG	CGGACAGCCA	CAGCATGGAA	TTAGGATCTG	CCAGTCACAG	CACGACCTAC	4980
	AATAATCTGT	CGAGAATAAA	TAATGACGGC	ATTGTCGAGC	TGCTACACAA	ACATTTTCGAT	5040
40	GCGGCATTAC	CAGCAAGCAG	TGCCAAACGT	CTTGGTGAAA	TGATGAATAA	CGATCCGGCA	5100
	CTGAAAGATA	TTATTAAGCA	GCTGCAAAGT	ACGCCGTTCA	GCAGCGCCAG	CGTGTCGATG	5160
	GAGCTGAAAG	ATGGTCTGCG	TGAGCAGACG	GAAAAAGCAA	TACTGGACGG	TAAGGTCGGT	5220
45	CGTGAAGAAG	TGGGAGTACT	TTCCAGGAT	CGTAACAACT	TGCGTGTTAA	ATCGGTCAGC	5280
	GTCAGTCAGT	CCGTCAGCAA	AAGCGAAGGC	TTCAATACCC	CAGCGCTGTT	ACTGGGGACG	5340
50	AGCAACAGCG	CTGCTATGAG	CATGGAGCGC	AACATCGGAA	CCATTAATTT	TAAATACGGC	5400
	CAGGATCAGA	ACACCCACG	GCGATTTACC	CTGGAGGGTG	GAATAGCTCA	GGCTAATCCG	5460
	CAGGTCGCAT	CTGCGCTTAC	TGATTTGAAG	AAGGAAGGGC	TGAAATGAA	GAGCTAA	5517

55

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 28 as follows:

60

- 17 -

Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr  
 1 5 10 15  
 5 Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser  
 20 25 30  
 Ser Ser Ser Ser Pro Gln Asn Ala Ala Ser Leu Ala Ala Glu Gly  
 35 40 45  
 10 Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala  
 50 55 60  
 15 Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg  
 65 70 75 80  
 Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln  
 85 90 95  
 20 ~~Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala~~  
 100 105 110  
 Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala  
 115 120 125  
 25 Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met  
 130 135 140  
 30 Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro  
 145 150 155 160  
 Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln  
 165 170 175  
 35 Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp  
 180 185 190  
 Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile  
 195 200 205  
 40 Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala  
 210 215 220  
 45 Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln  
 225 230 235 240  
 Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro  
 245 250 255  
 50 Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys  
 260 265 270  
 Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln  
 275 280 285  
 55 Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val  
 290 295 300  
 60 Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro  
 305 310 315 320  
 Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys  
 325 330 335  
 65 Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln  
 340 345 350



- 19 -

Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr  
 690 695 700  
 5 Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly  
 705 710 715 720  
 Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu  
 725 730 735  
 10 Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val  
 740 745 750  
 Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu  
 755 760 765  
 15 Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly  
 770 775 780  
 20 ~~Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe~~  
 785 790 795 800  
 Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu  
 805 810 815  
 25 Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His  
 820 825 830  
 Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln  
 835 840 845  
 30 Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys  
 850 855 860  
 35 Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser  
 865 870 875 880  
 His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln  
 885 890 895  
 40 Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro  
 900 905 910  
 Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met  
 915 920 925  
 45 Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys  
 930 935 940  
 50 Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val  
 945 950 955 960  
 Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr  
 965 970 975  
 55 Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His  
 980 985 990  
 Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly  
 995 1000 1005  
 60 Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro  
 1010 1015 1020  
 65 Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His  
 1025 1030 1035 1040

- 20 -

Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu  
 1045 1050 1055  
 5 Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val  
 1060 1065 1070  
 Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly  
 1075 1080 1085  
 10 Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu  
 1090 1095 1100  
 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu  
 1105 1110 1115 1120  
 Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp  
 1125 1130 1135  
 20 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro  
 1140 1145 1150  
 Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val  
 1155 1160 1165  
 25 Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser  
 1170 1175 1180  
 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe  
 1185 1190 1195 1200  
 30 Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr  
 1205 1210 1215  
 35 Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp  
 1220 1225 1230  
 Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val  
 1235 1240 1245  
 40 Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu  
 1250 1255 1260  
 Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser  
 1265 1270 1275 1280  
 Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val  
 1285 1290 1295  
 50 Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly  
 1300 1305 1310  
 Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly  
 1315 1320 1325  
 55 Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile  
 1330 1335 1340  
 Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys  
 1345 1350 1355 1360  
 60 Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile  
 1365 1370 1375

- 21 -

Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly  
 1380 1385 1390  
 Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro  
 1395 1400 1405  
 Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu  
 1410 1415 1420  
 Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr  
 1425 1430 1435 1440  
 Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn  
 1445 1450 1455  
 Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser  
 1460 1465 1470  
 Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg  
 1475 1480 1485  
 Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn  
 1490 1495 1500  
 Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala  
 1505 1510 1515 1520  
 Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly  
 1525 1530 1535  
 Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu  
 1540 1545 1550  
 Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu  
 1555 1560 1565  
 Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys  
 1570 1575 1580  
 His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu  
 1585 1590 1595 1600  
 Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His  
 1605 1610 1615  
 Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg  
 1620 1625 1630  
 Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser  
 1635 1640 1645  
 Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser  
 1650 1655 1660  
 Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp  
 1665 1670 1675 1680  
 Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn  
 1685 1690 1695  
 Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro  
 1700 1705 1710  
 Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu  
 1715 1720 1725

- 22 -

Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val  
 1730 1735 1740  
 5 Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser  
 1745 1750 1755 1760  
 Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu  
 1765 1770 1775  
 10 Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile  
 1780 1785 1790  
 Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg  
 1795 1800 1805  
 15 Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser  
 1810 1815 1820  
 20 Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser  
 1825 1830 1835

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

25 The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 29 as follows:

ATGACATCGT CACAGCAGCG GGTGAAAGG TTTTACAGT ATTTCTCCGC CGGGTGTAAG 60  
 30 ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG 120  
 GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG 180  
 GCTGACCCAC AAACCTCAAT AACCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG 240  
 35 GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT 300  
 CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTCGCG ATATCGTTAG CGGCTTCATC 360  
 40 GAACATGCGG CAGAAGTGC TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA 420

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 30 as follows:  
 45

Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser  
 1 5 10 15  
 50 Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu  
 20 25 30  
 Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His  
 35 40 45  
 55 Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln  
 50 55 60



- 23 -

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met  
 65 70 75 80  
 5 Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val  
 85 90 95  
 Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe  
 100 105 110  
 10 Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu  
 115 120 125  
 Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala  
 130 135  
 15

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID.

20 No. 31 as follows:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
 1 5 10 15  
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser  
 20 25 30  
 25 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met  
 35 40 45  
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala  
 50 55 60  
 30 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val  
 65 70 75 80  
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
 85 90 95  
 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met  
 100 105 110  
 35 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
 115 120 125  
 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
 130 135 140  
 40 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
 145 150 155 160  
 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
 165 170 175  
 Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
 180 185 190

- 24 -

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
 195 200 205  
 Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
 210 215 220  
 Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser  
 225 230 235 240  
 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp  
 245 250 255  
 Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val  
 260 265 270  
 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
 275 280 285  
 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
 290 295 300  
 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
 305 310 315 320  
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
 325 330 335  
 Asn Gln Ala Ala Ala  
 340

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "Pseudomonas syringae pv. syringae Harpin<sub>PS</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 32 as follows:

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTG 60  
 GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC 120  
 GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA 180  
 AACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC 240  
 ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG 300

- 25 -

GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC 360  
 AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420  
 GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480  
 AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540  
 5 GAAACGGCTG CGTTCCGTTC GGCACCTGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG 600  
 AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC 660  
 AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720  
 GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780  
 TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCGCAGAC CGGTACGTCG 840  
 10 GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900  
 GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960  
 GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020  
 GCCTGA 1026

15 Another potentially suitable hypersensitive response elicitor from  
*Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817,  
 which is hereby incorporated by reference. The protein has a nucleotide sequence of  
 SEQ. ID. No. 33 as follows:

20 TCCAATTTCG TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG 60  
 CTGAGTGC GC AGATTTTCGT GATAAGGGTG TGGTACTGGT CATGTGTTGGT CATTTCAGG 120  
 CCTCTGAGTG CCGTGCGGAG CAATACCAGT CTTCTGCTG GCGTGTGCAC ACTGAGTCGC 180  
 25 AGGCATAGGC ATTTTCAGTTC CTGCGTTGG TTGGGCATAT AAAAAAAGGA ACTTTTAAAA 240  
 ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG 300  
 30 AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC 360  
 TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCCC TTAGCGAGGT AACGCAGCAT 420  
 GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACCACG CCACTCGATT TTTCGGCGCT 480  
 35 AAGCGGCAAG AGTCCTCAAC CAAACAGTTC CGGCGAGCAG AACACTCAGC AAGCGATCGA 540  
 CCCGAGTGCA CTGTTGTTTC GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCCCGA 600  
 40 CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC 660  
 TAAATTGATC AGTGCAATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA 720  
 GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAAACA ACGGCGGGCT 780

- 26 -

5 CCGTACACCG TCGGCCGATA GCGGGGGCGG CCGTACACCG GATGCGACAG GTGGCGGCGG 840  
 CCGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG 900  
 TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC 960  
 CACTGCAACA GGCAGTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA 1020  
 10 CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTTCTA CCGAGCAAGC 1080  
 CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGGTCGGC GCTGGCGAAG TCTTTGACGG 1140  
 CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA 1200  
 15 GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTGAAG AATGTGAACC TGGGTGAGAA 1260  
 CGAGGTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT 1320  
 20 GCATGCCCAG AACGTCGGTG AAGACCTGAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT 1380  
 CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT 1440  
 CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAAGGCC GACGATTTTCG GCACGATGGT 1500  
 25 TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC 1560  
 TAACCACGGC AAGTTCGCCC TGGTGAAGG CGACAGTGAC GATCTGAAGC TGGCAACGGG 1620  
 30 CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680  
 CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAGGG GGTGGACTC 1729

35 This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 34 as follows:

40 Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu  
 1 5 10 15  
 Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly  
 20 25 30  
 45 Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly  
 35 40 45  
 Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val  
 50 55 60  
 Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile  
 65 70 75 80  
 55 Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr  
 85 90 95

- 27 -

	Asn	Ser	Asn	Lys	Lys	Gln	Asp	Thr	Asn	Gln	Glu	Gln	Pro	Asp	Ser	Gln	
				100					105					110			
5	Ala	Pro	Phe	Gln	Asn	Asn	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ala	Asp	Ser	
			115					120					125				
	Gly	Gly	Gly	Gly	Thr	Pro	Asp	Ala	Thr	Gly	Gly	Gly	Gly	Gly	Asp	Thr	
			130				135						140				
10	Pro	Ser	Ala	Thr	Gly	Gly	Gly	Gly	Asp	Thr	Pro	Thr	Ala	Thr	Gly		
	145					150				155						160	
	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Thr	Pro	Thr	Ala	Thr	Gly	Gly	Gly	
					165					170					175		
15	Ser	Gly	Gly	Thr	Pro	Thr	Ala	Thr	Gly	Gly	Gly	Glu	Gly	Gly	Val	Thr	
				180					185					190			
20	Pro	Gln	Ile	Thr	Pro	Gln	Leu	Ala	Asn	Pro	Asn	Arg	Thr	Ser	Gly	Thr	
			195					200					205				
	Gly	Ser	Val	Ser	Asp	Thr	Ala	Gly	Ser	Thr	Glu	Gln	Ala	Gly	Lys	Ile	
		210					215					220					
25	Asn	Val	Val	Lys	Asp	Thr	Ile	Lys	Val	Gly	Ala	Gly	Glu	Val	Phe	Asp	
	225					230					235					240	
	Gly	His	Gly	Ala	Thr	Phe	Thr	Ala	Asp	Lys	Ser	Met	Gly	Asn	Gly	Asp	
					245					250					255		
30	Gln	Gly	Glu	Asn	Gln	Lys	Pro	Met	Phe	Glu	Leu	Ala	Glu	Gly	Ala	Thr	
				260					265					270			
35	Leu	Lys	Asn	Val	Asn	Leu	Gly	Glu	Asn	Glu	Val	Asp	Gly	Ile	His	Val	
			275					280					285				
	Lys	Ala	Lys	Asn	Ala	Gln	Glu	Val	Thr	Ile	Asp	Asn	Val	His	Ala	Gln	
		290					295					300					
40	Asn	Val	Gly	Glu	Asp	Leu	Ile	Thr	Val	Lys	Gly	Glu	Gly	Gly	Ala	Ala	
	305					310					315					320	
	Val	Thr	Asn	Leu	Asn	Ile	Lys	Asn	Ser	Ser	Ala	Lys	Gly	Ala	Asp	Asp	
					325					330					335		
45	Lys	Val	Val	Gln	Leu	Asn	Ala	Asn	Thr	His	Leu	Lys	Ile	Asp	Asn	Phe	
				340					345					350			
50	Lys	Ala	Asp	Asp	Phe	Gly	Thr	Met	Val	Arg	Thr	Asn	Gly	Gly	Lys	Gln	
		355						360					365				
	Phe	Asp	Asp	Met	Ser	Ile	Glu	Leu	Asn	Gly	Ile	Glu	Ala	Asn	His	Gly	
		370					375					380					
55	Lys	Phe	Ala	Leu	Val	Lys	Ser	Asp	Ser	Asp	Asp	Leu	Lys	Leu	Ala	Thr	
	385					390				395						400	
	Gly	Asn	Ile	Ala	Met	Thr	Asp	Val	Lys	His	Ala	Tyr	Asp	Lys	Thr	Gln	
					405					410					415		

- 28 -

Ala Ser Thr Gln His Thr Glu Leu  
420

5

This protein or polypeptide is about 42.9 kDa.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ.

10 ID. No. 35 as follows:

	Met	Ser	Val	Gly	Asn	Ile	Gln	Ser	Pro	Ser	Asn	Leu	Pro	Gly	Leu	Gln	
	1				5					10					15		
15	Asn	Leu	Asn	Leu	Asn	Thr	Asn	Thr	Asn	Ser	Gln	Gln	Ser	Gly	Gln	Ser	
				20					25					30			
	Val	Gln	Asp	Leu	Ile	Lys	Gln	Val	Glu	Lys	Asp	Ile	Leu	Asn	Ile	Ile	
			35					40					45				
	Ala	Ala	Leu	Val	Gln	Lys	Ala	Ala	Gln	Ser	Ala	Gly	Gly	Asn	Thr	Gly	
			50				55					60					
20	Asn	Thr	Gly	Asn	Ala	Pro	Ala	Lys	Asp	Gly	Asn	Ala	Asn	Ala	Gly	Ala	
	65					70					75				80		
	Asn	Asp	Pro	Ser	Lys	Asn	Asp	Pro	Ser	Lys	Ser	Gln	Ala	Pro	Gln	Ser	
					85					90					95		
25	Ala	Asn	Lys	Thr	Gly	Asn	Val	Asp	Asp	Ala	Asn	Asn	Gln	Asp	Pro	Met	
				100					105					110			
	Gln	Ala	Leu	Met	Gln	Leu	Leu	Glu	Asp	Leu	Val	Lys	Leu	Leu	Lys	Ala	
			115					120					125				
	Ala	Leu	His	Met	Gln	Gln	Pro	Gly	Gly	Asn	Asp	Lys	Gly	Asn	Gly	Val	
			130				135					140					
30	Gly	Gly	Ala	Asn	Gly	Ala	Lys	Gly	Ala	Gly	Gly	Gln	Gly	Gly	Leu	Ala	
	145					150					155				160		
	Glu	Ala	Leu	Gln	Glu	Ile	Glu	Gln	Ile	Leu	Ala	Gln	Leu	Gly	Gly	Gly	
				165						170				175			
35	Gly	Ala	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Gly	Val	Gly	Gly	Ala	Gly	Gly	
				180					185					190			
	Ala	Asp	Gly	Gly	Ser	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Ala	Asn	Gly	Ala	
			195					200					205				
	Asp	Gly	Gly	Asn	Gly	Val	Asn	Gly	Asn	Gln	Ala	Asn	Gly	Pro	Gln	Asn	
			210				215					220					

- 29 -

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240  
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
 245 250 255  
 5 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
 260 265 270  
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
 275 280 285  
 10 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
 290 295 300  
 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
 305 310 315 320  
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
 325 330 335  
 15 Gln Ser Thr Ser Thr Gln Pro Met  
 340

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ.  
ID. No. 36 as follows:

ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC 60  
 20 AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC 120  
 GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC 180  
 GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC 240  
 AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC 300  
 GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA 360  
 25 GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG 420  
 GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC 480  
 GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC 540  
 GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT 600  
 GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC 660  
 30 GGCCCGCAGA ACGCAGGCGA TGTC AACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC 720  
 CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG 780  
 ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC 840  
 GGCAACGCCT CGCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT 900

- 30 -

GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC 960  
 GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG 1020  
 ACGCAGCCGA TGTA 1035

5 Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted  
 10 via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *glycines* has an amino acid sequence corresponding to SEQ. ID. No. 37 as follows:

15 Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala  
 1 5 10 15  
 Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr  
 20 25

20

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. *glycines*. It matches with fimbrial subunit proteins determined in other  
 25 *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 38 as follows:

30

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln  
 1 5 10 15  
 Leu Leu Ala Met  
 20

35

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*



subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not  
5 Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from  
10 *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and  
15 Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992), Baillreul et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A  
20 Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

25 Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

30 The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture

- 32 -

supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia amylovora* hypersensitive response elicitor. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, or an internal fragment of the amino acid sequence of SEQ. ID. No. 23. The C-terminal fragment of the amino acid

- 33 -

sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable  
5 fragments can be identified in accordance with the present invention.

Another example of a useful fragment of a hypersensitive response elicitor which fragment does not itself elicit a hypersensitive response is the protein fragment containing amino acids 190 to 294 of the amino acid sequence (SEQ. ID. No. 31) for the *Pseudomonas syringae* pv. *syringae* hypersensitive response elicitor.  
10 This fragment is useful in imparting disease resistance and enhancing plant growth.

Yet another example of a useful fragment of a hypersensitive response elicitor is the peptide having an amino acid sequence corresponding to SEQ. ID. No. 39. This peptide is derived from the hypersensitive response eliciting glycoprotein of *Phytophthora megasperma* and enhances plant growth.

15 Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide  
20 may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The fragment of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional  
25 techniques. Typically, the fragment of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein fragment, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment,  
30 and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the fragment is separated by centrifugation. The supernatant fraction containing the fragment is subjected to gel filtration in an

- 34 -

appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A

- 35 -

Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

- 36 -

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the  $P_R$  and  $P_L$  promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

- 37 -

Once the isolated DNA molecule encoding the fragment of a hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the fragment of a hypersensitive response elicitor polypeptide or protein which does not elicit a hypersensitive response in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the fragment to impart disease resistance, enhance growth, and/or control insects. Alternatively, these fragments of a hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a fragment of a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

- 38 -

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated fragment or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the fragment. In the latter embodiment, the fragment can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response. Such bacteria must be capable of secreting or exporting the fragment so that the fragment can contact plant or plant seed cells. In these embodiments, the fragment is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of



- 39 -

pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention:

- 5 *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

- 10 With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds  
15 germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed  
20 germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

- Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present  
25 invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from  
30 releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

- 40 -

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding  
5 pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect  
10 pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots,  
15 propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the fragment of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant  
20 seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the fragment of the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application  
25 procedures can be envisioned by those skilled in the art provided they are able to effect contact of the fragment with cells of the plant or plant seed. Once treated with the fragment of the hypersensitive response elicitor of the present invention, the seeds  
30 can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the fragment of the hypersensitive response elicitor protein or polypeptide or whole elicitors to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

- 41 -

The fragment of the hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the fragment can be applied separately to plants with other materials being applied at different times.

5 A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains  
10 greater than 500 nM of the fragment.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

15 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response eliciting fragment can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

20 In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a fragment of a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such a fragment are produced according to procedures well known in the art.

25 The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby  
30 incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic

- 42 -

transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

*Agrobacterium* is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy

- 43 -

root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated. Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

- 44 -

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves  
5 can be cultivated in accordance with conventional procedure with the presence of the gene encoding the fragment of the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using  
10 conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the  
15 polypeptide or protein fragment.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a fragment of a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including  
20 a fragment of a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the fragment of a  
25 hypersensitive response elicitor in accordance with the present invention to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

## EXAMPLES

### 30 Example 1 - Bacterial Strains and Plasmids

*Escherichia coli* strains used in the following examples include DH5 $\alpha$  and BL21(DE3) purchased from Gibco BRL (Grand Island, N.Y.) and Stratagene

- 45 -

(La Jolla, CA), respectively. The pET28(b) vector was purchased from Novagen (Madison, WI). Eco DH5 $\alpha$ /2139 contained the complete *hrpN* gene. The 2139 construct was produced by D. Bauer at Cornell University. The *hrpN* gene was cleaved from the 2139 plasmid by restriction enzyme digestion with HindIII, then purified from an agarose gel to serve as the DNA template for PCR synthesis of truncated *hrpN* clones. These clones were subsequently inserted into the (His)<sub>6</sub> vector pET28(b) which contained a Kan<sup>r</sup> gene for selection of transformants.

### **Example 2 - DNA Manipulation**

Restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, IN) or Gibco BRL. T4 DNA ligase, Calf Intestinal Alkaline Phosphatase (CIAP), and PCR Supermix<sup>TM</sup> were obtained from Gibco BRL. The QIAprep Spin Miniprep Kit, the Qiagen Plasmid Mini Kit, and the QIAquick PCR Purification Kit were purchased from Qiagen (Hilden, Germany). The PCR primers were synthesized by Lofstrand Labs Limited (Gaithersburg, MD). The oligopeptides were synthesized by Bio-Synthesis, Inc. (Lewisville, TX). All DNA manipulations such as plasmid isolation, restriction enzyme digestion, DNA ligation, and PCR were performed according to standard techniques (Sambrook, et al., Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989)) or protocols provided by the manufacturer.

### **Example 3 - Fragmentation of *hrpN* Gene**

A series of N-terminal and C-terminal truncated *hrpN* genes and internal fragments were generated via PCR (Fig. 1). The full length *hrpN* gene was used as the DNA template and 3' and 5' primers were designed for each truncated clone (Fig. 2). The 3' primers contained an NdeI enzyme cutting site which contained the start codon ATG (methionine) and the 5' primers contained the stop codon TAA and a HindIII enzyme cutting site for ligation into the pET28(b) vector. PCR was carried out in 0.5 ml tubes in a GeneAmp<sup>TM</sup> 9700 (Perkin-Elmer, Foster City, CA). 45  $\mu$ l of Supermix<sup>TM</sup> (Life Technology, Gaithersburg, MD) were mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and deionized

- 46 -

H<sub>2</sub>O to a final volume of 50 µl. After heating the mixture at 95°C for 2 min, the PCR was performed for 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1.5 min. The PCR products were verified on a 6% TBE gel (Novex, San Diego, CA). Amplified DNA was purified with the QIAquick PCR purification kit, digested with

5 Nde I and Hind III at 37°C for 5 hours, extracted once with phenol:chloroform:isoamylalcohol (25:25:1) and precipitated with ethanol. 5 µg of pET28(b) vector DNA were digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with CIAP treatment to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with

10 the QIAquick PCR purification kit and directly used for ligation. Ligation was carried out at 14-16°C for 5-12 hours in a 15 µl mixture containing ca. 200 ng of digested pET28(b), 30 ng of targeted PCR fragment, and 1 unit T4 DNA ligase. 5 - 7.5 µl of ligation solution were added to 100 µl of DH5α competent cells in a 15 ml Falcon tube and incubated on ice for 30 min. After a heat shock at 42°C for 45 seconds, 0.9

15 ml SOC solution or 0.45 ml LB media were added to each tube and incubated at 37°C for 1 hour. 20, 100, and 200 µl of transformed cells were placed onto LB agar with 30 µg/ml of kanamycin and incubated at 37°C overnight. Single colonies were transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared from 2 ml of culture with the QIAprep Miniprep kit (QIAGEN, Hilden,

20 Germany). The DNA from the transformed cells was analyzed by restriction enzyme digestion or partial sequencing to verify the success of the transformations. Plasmids with the desired DNA sequence were transferred into the BL21 strain using the standard chemical transformation method as indicated above. A clone containing the full length harpin protein in the pET28(b) vector was generated as a positive control,

25 and a clone with only the pET28(b) vector was generated as a negative control.

#### **Example 4 - Expression of Hypersensitive Response Elicitor Truncated Proteins**

*Escherichia coli* BL21(DE3) strains containing the hrpN clones were

30 grown in Luria broth medium (5g/L Difco Yeast extract, 10 g/L Difco Tryptone, 5 g/L NaCl, and 1 mM NaOH) containing 30 µg/ml of kanamycin at 37°C overnight. The bacteria were then inoculated into 100 volumes of the same medium and grown at



- 47 -

37°C to an OD<sub>620</sub> of 0.6-0.8. The bacteria were then inoculated into 250 volumes of the same medium and grown at 37°C to an OD<sub>620</sub> of ca. 0.3 or 0.6-0.8. One millimolar IPTG was then added and the cultures grown at 19°C overnight (ca. 18 hours). Not all of the clones were successfully expressed using this strategy. Several of the clones had to be grown in Terrific broth (12 g/L Bacto Tryptone, 24 g/L Bacto yeast, 0.4% glycerol, 0.17 M KH<sub>2</sub>PO<sub>4</sub>, and 0.72 K<sub>2</sub>HPO<sub>4</sub>), and/or grown at 37°C after IPTG induction, and/or harvested earlier than overnight (Table 1).

Table 1: Expression of hypersensitive response elicitor truncated proteins

Fragment	amino acids (SEQ. ID. No. 23)	Growth medium	Induction O.D.	Expression temp.	Harvest time
1 (+ control)	1-403	LB	ca. 0.3 or 0.6-0.8	19°C or 25°C	16-18 hr
2 (+ control)	-	LB and TB	ca. 0.3 or 0.6-0.8	19 C and 37 C	16-18 hr
3	105-403	LB	0.6-0.8	19°C	16-18 hr
4	169-403	TB	ca. 0.3	19°C	16-18 hr
5	210-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
6	257-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
7	343-403	LB	ca. 0.3	19°C	5 hr
8	1-75	TB	ca. 0.3	37°C	16-18 hr
9	1-104	TB	ca. 0.3	37°C	16-18 hr
10	1-168	TB	ca. 0.3	37°C	16-18 hr
11	1-266	LB	ca. 0.3	37°C	4 hr
12	1-342	LB	0.6-0.8	19°C	16-18 hr
13	76-209	LB	ca. 0.3	37°C	5 hr
14	76-168	TB or LB	ca. 0.3	37°C	3 hr or 16-18 hr
15	105-209	M9ZB	ca. 0.3	37°C	3 hr
16	169-209	no expression			
17	105-168	LB	ca. 0.3	37°C	3-5 hr
18	99-209	LB	ca. 0.3	37°C	3 hr
19	137-204	LB	ca. 0.3	37°C	3 hr
20	137-180	LB	ca. 0.3	37°C	16-18 hr.
21	105-180	LB	ca. 0.3	37°C	3 hr
22	150-209	no expression			
23	150-180	no expression			

#### Example 5 - Small Scale Purification of Hypersensitive Response Elicitor Truncated Proteins (Verification of Expression)

A 50 ml culture of a hrpN clone was grown as above to induce expression of the truncated protein. Upon harvesting of the culture, 1.5 ml of the cell

- 48 -

suspension were centrifuged at 14,000 rpm for 5 minutes, re-suspended in urea lysis buffer (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, and 0.01 M Tris -- pH 8.0), incubated at room temperature for 10 minutes, then centrifuged again at 14,000 rpm for 10 minutes, and the supernatant saved. A 50 µl aliquot of a 50% slurry of an equilibrated (His)<sub>6</sub>-  
5 binding nickel agarose resin was added to the supernatant and mixed at 4°C for one hour. The nickel agarose was then washed three times with urea washing buffer (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, and 0.01 M Tris -- pH 6.3), centrifuging at 5,000 rpm for five minutes between washings. The protein was eluted from the resin with 50 µl of urea elution buffer (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, 0.01 M Tris, and 0.1 M EDTA -- pH 6.3).  
10 The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression.

#### **Example 6 - Induction of HR in Tobacco**

A 1.5 ml aliquot from the 50 ml cultures grown for small scale  
15 purification of the truncated proteins was centrifuged at 14,000 rpm for four minutes and re-suspended in an equal volume of 5 mM potassium phosphate buffer, pH 6.8. The cell suspension was sonicated for ca. 30 seconds then diluted 1:2 and 1:10 with phosphate buffer. Both dilutions plus the neat cell lysate were infiltrated into the fourth to ninth leaves of 10-15 leaf tobacco plants by making a hole in single leaf  
20 panes and infiltrating the bacterial lysate into the intercellular leaf space using a syringe without a needle. The HR response was recorded 24-48 hr post infiltration. Tobacco (*Nicotiana tabacum* v. Xanthi) seedlings were grown in an environmental chamber at 20-25°C with a photoperiod of 12-h light /12-h dark and ca. 40% RH. Cell lysate was used for the initial HR assays (in order to screen the truncated proteins  
25 for HR activity) as the small scale urea purification yielded very little protein which was denatured due to the purification process.

#### **Example 7 - Large Scale Native Purification of Hypersensitive Response Elicitor Truncated Proteins for Comprehensive Biological Activity Assays**

30 Six 500 ml cultures of a hrpN clone were grown as described earlier to induce expression of the truncated protein. Upon harvesting of the culture, the cells were centrifuged at 7,000 rpm for 5 minutes, re-suspended in imidazole lysis buffer (5

- 49 -

mM imidazole, 0.5 M NaCl, 20 mM Tris) plus Triton X-100 at 0.05% and lysozyme at 0.1 mg/ml, incubated at 30°C for 15 minutes, sonicated for two minutes, centrifuged again at 15,000 rpm for 20 minutes, and the supernatant was saved. A 4 ml aliquot of a 50% slurry of an equilibrated (His)<sub>6</sub>-binding nickel agarose resin was added to the supernatant and mixed at 4°C for ca. four hours. The nickel agarose was then washed three times with imidazole washing buffer (20 mM imidazole, 0.5 M NaCl, and 20 mM Tris), centrifuging at 5,000 rpm for five minutes between washings, then placed in a disposable chromatography column. The column was centrifuged at 1100 rpm for one minute to remove any residual wash buffer and then the protein was eluted from the resin with 4 ml of imidazole elution buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris) by incubating the column with the elution buffer for ten minutes at room temperature and then centrifuging the column at 1100 rpm for one minute. The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression. The concentration of the proteins was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker.

**Example 8 - Large Scale Urea Purification of Hypersensitive Response Elicitor Truncated Proteins For Comprehensive Biological Activity Assay**

The procedure was the same as the large scale native purification except that urea lysis buffer, washing buffer, and elution buffer were used, and the cells were not sonicated as in the native purification. After purification, the protein was renatured by dialyzing against lower and lower concentrations of urea over an eight hour period, then dialyzing overnight against 10 mM Tris/20 mM NaCl. The renaturing process caused the N-terminal proteins to precipitate. The precipitated 1-168 protein was solubilized by the addition of 100 mM Tris-HCl at pH 10.4 then heating the protein at 30°C for ca. one hour. The concentration of the protein was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker. The 1-75 and 1-104 protein fragments were not successfully solubilized using this strategy so they were sonicated in 100 mM Tris-HCl at pH 10.4 to solubilize as much of the protein as possible and expose the active sites of the protein for the biological activity assays.

**Example 9 – Induction of Growth Enhancement (GE)**

Sixty tomato (*Lycopersicon spp.* cv. Marglobe) seeds were soaked overnight in 10 and 20 µg/ml of the truncated protein diluted with 5mM potassium phosphate buffer, pH 6.8. The next morning, the sixty seeds were sewn in three pots and 12-15 days later and again 18-20 days later the heights of the 10 tallest tomato plants per pot were measured and compared with the heights of the control plants treated only with phosphate buffer. Analyses were done on the heights to determine if there was a significant difference in the height of the plants treated with the truncated proteins compared with the buffer control, and thereby determine whether the proteins induced growth enhancement.

**Example 10 – Induction of Systemic Acquired Resistance (SAR)**

Three tobacco (*Nicotiana tabacum* cv. Xanthi) plants with 8-12 leaves (ca. 75 day old plants) were used in the assay. One leaf of the tobacco plants was covered up and the rest of the leaves were sprayed with ca. 50 ml of a 20 µg/ml solution of the truncated proteins diluted with 5mM potassium phosphate buffer. Five to seven days later two leaves (the unsprayed leaf and the sprayed leaf opposite and just above the unsprayed leaf) were inoculated with 20 µl of a 1.8 µg/ml solution of TMV along with a pinch of diatomaceous earth by rubbing the mixture along the top surface of the leaves. The TMV entered the plants through tiny lesions made by the diatomaceous earth. Ca. 3-4 days post TMV inoculation, the number of TMV lesions was counted on both leaves compared with the number of lesions on the negative control buffer treated leaves. Analyses were done to determine the efficacy of reducing the number of TMV lesions by the protein fragments compared to the buffer control. Percentage of efficacy was calculated as:  $\text{Reduction in TMV lesions (\% efficacy)} = 100 \times (1 - \text{mean \# of lesions on treated leaves} / \text{mean \# of lesions on buffer control leaves})$ .

- 51 -

**Example 11 - Expression of Hypersensitive Response Elicitor Truncated Proteins**

The small scale expression and purification of the fragment proteins was done to screen for expression and HR activity (Table 2).

Table 2

Expression and HR activity of hypersensitive response elicitor truncated proteins (small scale screening)

Fragment #	Amino Acids (SEQ. ID. No. 23)	Expression	HR activity
1(+control)	1-403	+	+
2(- control)	-	background protein only	-
3	105-403	+	+
4	169-403	+	-
5	210-403	+	-
6	267-403	+	-
7	343-403	+/-	-
8	1-75	+	-
9	1-104	+	+/-
10	1-168	+	+
11	1-266	+	+
12	1-342	+	+
13	76-209	+	+
14	76-168	+	-
15	105-209	+	+
16	169-209	-	-
17	105-168	+	-
18	99-209	+	+
19	137-204	+	+
20	137-180	+	+
21	105-180	+	+
22	150-209	-	-
23	150-180	-	-

All of the cloned fragment proteins were expressed at varying levels except for three small fragments (amino acids 169-209, 150-209, and 150-180). Fragments 210-403 and 267-403 were expressed very well, yielding a high concentration of protein from a small scale purification, resulting in a substantial protein band on SDS gel electrophoresis. Other fragments (such as a.a. 1-168 and 1-104) produced much less protein, resulting in faint protein bands upon electrophoresis. It was difficult to determine whether fragment 343-403, the smallest C-terminal protein, was expressed, as there were several background proteins apparent on the gel, in addition to the suspected 343-403 protein. The positive and negative control proteins, consisting of

- 52 -

the full length hypersensitive response elicitor protein and only background proteins, respectively, were tested for expression and HR activity as well.

The large scale expression and purification of the fragment proteins was done to determine the level of expression and titer of the HR activity (Table 3).

Table 3

Expression level and HR titer of hypersensitive response elicitor truncated proteins (large sale purification)

Fragment #	Amino acids (SEQ. ID. No. 23)	Expression	HR titer
1(+ control)	1-403	3.7 mg/ml	5-7 µg/ml
2 (- control)	-	-	1:2 dilution
4	169-403	2 mg/ml	-
5	210-403	5 mg/ml	-
6	267-403	4 mg/ml	-
7	343-402	200µg/ml	-
8	1-75	50µg/ml	-
9	1-104	50µg/ml	3 µg/ml (1:16 dilution)
10	1-168	1 mg/ml	1 µg/ml
13	76-209	2.5 mg/ml	5 µg/ml
14	76-168	2 mg/ml	-
15	105-209	5 mg/ml	5-10µg/ml
17	105-168	250µg/ml	-
19	137-204	3.6 mg/ml	3.5 µg/ml
20	137-180	250 µg/ml	16 µg/ml

The truncated proteins deemed to be the most important in characterizing the hypersensitive response elicitor were chosen for large scale expression. The positive control (full length hypersensitive response elicitor) was expressed at a relatively high level at 3.7 mg/ml. All of the C-terminal proteins were expressed at relatively high levels from 2-5 mg/ml, except for fragment 343-403 as discussed earlier. The N-terminal fragments were expressed very well also; however, during the purification process, the protein precipitated and very little was resolubilized. The concentrations in Table 3 reflect only the solubilized protein. The internal fragments were expressed in the range of 2-3.6 mg/ml. It was extremely difficult to determine the concentration of fragment 105-168 (it was suspected that the concentration was much higher than indicated), as the protein bands on the SDS gel were large, but poorly stained. The

- 53 -

negative control contained several background proteins as expected, but no obviously induced dominant protein.

### **Example 12 - Induction of HR in Tobacco**

5                   The full length positive control protein elicited HR down to only 5-7 µg/ml. The negative control (pET 28) imidazole purified "protein" - which contained only background proteins - elicited an HR response down to the 1:2 dilution, which lowered the sensitivity of the assay as the 1:1 and 1:2 dilutions could  
10 not be used. This false HR was likely due to an affinity of the imidazole used in the purification process to bind to one or several of the background proteins, thereby not completely dialyzing out. Imidazole at a concentration of ca. 60 mM did elicit a false HR response.

                  One definitive domain encompassing a small internal region of the  
15 protein from a.a. 137-180 (SEQ. ID. No. 23), a mere 44 a.a, is identified as the smallest HR domain. The other potential HR domain is thought to be located in the N-terminus of the protein from a.a. 1-104 (possibly a.a. 1-75) (SEQ. ID. No. 23). It was difficult to confirm or narrow down the N-terminus HR domain due to the difficulties encountered in purifying these fragment proteins. The N-terminus  
20 fragment proteins had to be purified with urea as no protein was recovered when the native purification process was used. Consequently, these proteins precipitated during the renaturing process and were difficult or nearly impossible to get back into solution, thereby making it hard to run the proteins through the HR assay, as only soluble protein is able to elicit HR. Difficulty narrowing the N-terminus HR domain  
25 was only compounded by the fact that the negative control elicited false HR at the low dilution levels thereby reducing the sensitivity of the assay.

                  Surprisingly, when the internal HR domain was cleaved between a.a. 168 and 169 (fragments 76-168 and 105-168) (SEQ. ID. No. 23) the fragment lost its HR activity. This suggests that the HR activity of fragment 1-168 (SEQ. ID. No. 23)  
30 should not be attributed to the internal HR domain, but rather to some other domain, leading to the assumption that there was likely a second HR domain to be found in the N-terminal region of the protein. However, as discussed earlier it was difficult to confirm this assumption.

- 54 -

The hypersensitive response elicitor C-terminus (a.a. 210-403 (SEQ. ID. No. 23)) did not contain an HR domain. It did not elicit HR at a detectable level using the current HR assay. Even the large C-terminal fragment from a.a. 169-403 (SEQ. ID. No. 23) did not elicit HR even though it contained part of the internal HR domain. As stated above, cleaving the protein between amino acids 168 and 169 (SEQ. ID. No. 23) causes a loss of HR activity.

Because some of the small cloned proteins with 61 a.a. or less were not expressed, several oligopeptides were synthesized with 30 a.a. to narrow down the functional region of the internal HR domain. The oligopeptides were synthesized within the range of a.a. 121-179 (SEQ. ID. No. 23). However, these oligos did not elicit HR. It was not expected that there would be an HR from oligos 137-166, 121-150, and 137-156 (SEQ. ID. No. 23) as these fragments did not contain the imperative amino acids 168 and 169 (SEQ. ID. No. 23). It was expected that the oligo 150-179 (SEQ. ID. No. 23) would elicit an HR. It is possible that 30 a.a. is too small for the protein to elicit any activity due to a lack of folding and, therefore, a lack of binding or that during the synthesis of the peptides important amino acids were missed (either in the process, or simply by the choice of which 30 amino acids to synthesize) and, therefore, the fragments would not be able to elicit HR.

### 20 **Example 13 – Induction of Plant Growth Enhancement (PGE)**

The C-terminal fragments enhanced the growth of tomato by 9% to 21%. The N-terminal fragments enhanced the growth of tomato by 4% to 13%. The internal fragments enhanced growth by 9% to 20%. The 76-209 fragment enhanced growth by 18% at a concentration of 60 µg/ml, but not at the typical 20 µg/ml. This was attributed to the inaccuracy of the quantification process (Table 4).



- 55 -

Table 4

Fragment #	Amino acids	PGE ht>buffer @ 10 µg/ml	PGE ht>buffer @ 20 µg/ml
1 (+ control)	1-403	12%	11%
2 (- control)	-	-3%	-2%
4	169-403	9%	12%
5	210-403	13%	14%
			16% @ 40µg/ml
6	267-403	21%	21%
			23% @ 40µg/ml
7	343-403	7%	7%
9	1-104	4%	8%
10	1-168	13%	5%
13	76-209	7%	4%
			18% @ 60µg/ml
14	76-168	18%	20%
15	105-209	14%	19%
17	105-168	19%	16%
19	137-204	11%	13%
20	137-180	--	9%

\*A height greater than 10% above the buffer control was necessary to pass the PGE assay.

The oligopeptides enhanced growth from 7.4% to 17.3% (Table 5).

Table 5

Fragment	Amino acids	Expression	HR titer	TMV efficacy	PGE ht>buffer
oligo	150-179	NA	-	72.9%	10.1%
oligo	137-166	NA	-	61.2%	12.0%
oligo	121-150	NA	-	60.0%	17.3%
oligo	137-156	NA	-	-87.7%	7.4%

The data suggests that there is more than one PGE domain, although the C-terminal and internal domains appear to be dominant over the N-terminal domain, as the N-terminal fragments enhanced growth the least amount.

#### **Example 14 – Induction of Systemic Acquired Resistance (SAR)**

All of the hypersensitive response elicitor fragments tested to date appear to have 60% efficacy or greater, except for the oligopeptide 137-156 (Tables 5 and 6).

- 56 -

Table 6

Fragment #	Amino acids	Efficacy of TMV control
1 (+ control)	1-403	84% & 72%
2 (- control)	-	40% & 31%
4	169-403	64% & 79%
5	210-403	77% and 78%
6	267-403	70% and 72%
9	1-104	82%
10	1-168	69%
13	76-209	44% and 84%
14	76-168	83% & 87%
15	105-209	57% and 67%
17	105-168	89%
19	137-204	89% & 77%
20	137-180	64% & 58%

5

These data suggest that there are multiple SAR domains within the protein.

#### **Example 15 – Relationship Between HR, PGE, and SAR**

10

It is clear that the hypersensitive response activity is separable from the plant growth enhancement activity. The C-terminal fragments clearly enhance the growth of tomato by ca. 20% at a concentration of only 20 µg/ml, but these same fragments were not able to elicit HR in tobacco, even at higher concentrations than 200 µg/ml. The SAR activity also appears to be separable from the HR activity. This finding is highly significant for future work on transgenic applications of the hypersensitive response elicitor technology. The fragments that induce PGE and/or SAR but do not elicit HR will be imperative for this technology, as constitutive expression of even low levels of an HR elicitor might kill a plant.

20

#### **Example 16 - Non-HR Eliciting Fragments Derived from the Hypersensitive Response Elicitor from *Pseudomonas syringae* pv. *syringae* Induce Resistance in Tobacco to TMV and Promote the Growth of Tomato**

25

To test whether non-HR eliciting fragments derived from HrpZ, the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, is able to induce disease resistance, several fragment constructs were made and the expressed

- 57 -

fragment proteins were tested for HR elicitation and disease resistance induction in tobacco and growth promotion in tomato.

The following segments of *hrpZ*, the gene encoding the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, were amplified by PCR using Pfu Turbo (Stratagene): Regions coding for amino acids 152-190, aa 152-294, aa 190-294, aa 301-341, and full length HrpZ (aa 1-341). The DNA fragments were cloned into pCAL-n (Stratagene) to create C-terminal fusion proteins to the calmodulin-binding peptide. pCAL-n was chosen, because the fusion protein could be easily and gently purified on calmodulin resin. The DNA was transformed into *E. coli* DH5 $\alpha$ , and the correct clones were identified. The clones were then transferred to *E. coli* BLR DE3 for protein expression. The bacteria were grown in Terrific Broth to an OD<sub>620</sub> of 0.8-1.0. Protein expression was then induced with IPTG and the bacteria were incubated for an additional 3 h. All of the HrpZ fragments were able to be expressed this way.

Amino acid fragments 152-294 and 190-294 were chosen for further analysis and characterization. It was expected that the fragment 152-294 contained a domain that elicited the HR, while fragment 190-294 contained no domain that elicited the HR. The cultures were spun down, and the bacteria resuspended in 40 ml of 10 mM Tris pH 8.0. Twenty  $\mu$ l of antifoam and 40  $\mu$ l of 200 mM PMSF were added, and the bacteria was sonicated to break open the cells. The bacterial debris was removed by centrifugation, and the supernatant was placed in a boiling water bath for 10 min. The precipitate was removed by centrifugation and the supernatant, a crude protein preparation, was retained for tests.

Fifteen  $\mu$ l of each supernatant was run on a gel and stained to determine if the protein was present. It was estimated that about five times as much of the 152-294 fragment was present as the 190-294 fragment. Several dilutions of each preparation were infiltrated into tobacco leaves on two plants for HR tests (Table 7). As shown in Table 7, the 152-294 fragment elicited an HR, but the 190-294 fragment did not.

- 58 -

Table 7

HR test results of HrpZ fragments

<u>HrpZ Fragment</u>	<u>Dilution of Fragment Preparation<sup>a</sup></u>			
	<u>1:2</u>	<u>1:5</u>	<u>1:25</u>	<u>1:125</u>
152-294	+,+ <sup>b</sup>	+,+	+,+	-, -
190-294	-,-	-,-	-,-	-,-

<sup>a</sup> The preparations were diluted with MilliQ water.<sup>b</sup> The results are indicated for each of two plants. +, HR; -, no HR.

The fragment preparations were then tested for inducing resistance to TMV and for growth enhancement. Due to the difference in concentration of the HrpZ fragments, the 152-294 preparation was diluted 40-fold and the 190-294 preparation was diluted 8-fold. The results showed that the 190-294 aa fragment reduced the number of TMV lesions by 85% in comparison to buffer controls (Table 8). In contrast, the 152-294 aa fragment reduced the number of TMV lesions by only 55%. As also shown in Table 8, plants treated with the 152-294 aa fragment grew 4.64% more than buffer treated plants, while plants treated with the 190-294 aa fragment grew 2.62% more than the buffer treated plants.

Table 8

HR test, TMV, and PGE test results

<u>HrpZ Fragment</u>	<u>HR elicitation<sup>a</sup></u>	<u>TMV (% efficacy)<sup>b</sup></u>	<u>PGE(% &gt; buffer ht)<sup>c</sup></u>
152-294	+	54.64	4.64
190-294	-	85.25	2.62

<sup>a</sup> +, elicits HR in tobacco leaves; -, no HR in tobacco leaves.<sup>b</sup> % reduction in TMV lesions in unsprayed leaf of tobacco.<sup>c</sup> % greater height than buffer sprayed plants.

The results of these tests show that amino acids 152-190 appear to be involved in HR elicitation, because their removal eliminated the ability to elicit the HR. Both fragment preparations achieved disease control and growth enhancement. Thus, the ability to elicit the HR is not the determining factor for reduction in TMV infection and growth enhancement.

- 59 -

**Example 17 - Use of 13 Amino Acid Peptide Derived from *Phytophthora megasperma* Stimulates Tomato Seedling Growth**

Parsley leaves develop a typical resistance reaction against the soybean pathogen *Phytophthora megasperma* comprising hypersensitive cell death, defense related gene activation, and phytoalexin formulation. Several years ago, a 42 kDa glycoprotein elicitor was purified from the fungal culture filtrate of *Phytophthora megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f.sp. glycinea Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991), which is hereby incorporated by reference). Then, an oligopeptide of 13 amino acid was identified within the 42 kDa glycoprotein. The 13 amino acids peptide appeared to have similar biological activity as that of the full length glycoprotein (42 kDa). It is sufficient to elicit a complex defense response in parsley cells including H<sup>+</sup>/Ca<sup>2+</sup> influxes, K<sup>+</sup>/Cl<sup>-</sup> effluxes, active oxygen production, SAR gene induction, and phytoalexin compound accumulation (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Response," Cell 78:449-460 (1994), which is hereby incorporated by reference).

To test if the 13 amino acid peptide derived from the 42 kDa protein also enhanced plant growth, 20 mg of the oligopeptide was synthesized from Biosynthesis Corp. The synthesized sequence of the peptide is NH<sub>2</sub>-Val-Trp-Asn-Gln-Pro-Val-Arg-Gly-Phe-Lys-Val-Tyr-Glu-COOH (SEQ. ID. No. 39). The synthesized peptide was resuspended in 10 ml of 5 mM potassium phosphate buffer and, then, diluted to 1 and 100 ng/ml with the same buffer. About 100 tomato seeds (variety, Marglobe) were submerged in 20 ml of peptide solution overnight. The soaked seeds were planted in an 8 inch pot with artificial soil. Seeds soaked in the buffer without the peptide were used as a control. After seedlings emerged and the first two true leaves fully expanded, the height of the tomato seedlings was recorded. The peptide was not able to elicit the HR in tobacco and other tested plants. However, it had a profound effect on plant growth promotion. Table 9 shows that tomato seedlings treated with the peptide increased 12.6 % in height, indicating that the fungal peptide derived from the 42 kDa glycoprotein can promote tomato seedling growth. Extended studies showed that the peptide also had similar growth

- 60 -

effect in other crops including tobacco. Similar growth promotion effects were achieved by plants sprayed with the peptide solution.

Table 9

5	Treatment	Height of seedlings (cm)					Average (cm) % Change	
	Buffer	6.0	6.0	6.0	5.5	5.5	5.55	-
		5.5	5.5	5.0	5.0	5.5		
10	Peptide Solution (100ng/ml)	6.5	6.0	6.5	6.5	6.5	6.25	12.6
		6.0	6.0	6.0	6.0	6.5		

15 Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

- 61 -

**WHAT IS CLAIMED:**

1. An isolated fragment of a hypersensitive response elicitor protein or polypeptide, wherein said fragment does not elicit a hypersensitive response but has other activity in plants.
2. An isolated fragment according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia Pseudomonas*, *Xanthomonas*, or *Phytophthora*.
3. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.
4. An isolated fragment according to claim 3, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.
5. An isolated fragment according to claim 4, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.
6. An isolated fragment according to claim 4, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.
7. An isolated fragment according to claim 4, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.

- 62 -

8. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

9. An isolated fragment according to claim 8, wherein the  
5 fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

10. An isolated DNA molecule encoding a fragment according to claim 1.

10 11. An isolated DNA molecule according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia Pseudomonas, Xanthomonas, or Phytophthora*.

12. An isolated DNA molecule according to claim 11, wherein the  
15 hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

13. An isolated DNA molecule according to claim 12, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino  
20 acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.

14. An isolated DNA molecule according to claim 12, wherein the  
25 fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.

15. An isolated DNA molecule according to claim 12, wherein the  
30 fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.



- 63 -

16. An isolated DNA molecule according to claim 12, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.

5

17. An isolated DNA molecule according to claim 11, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

18. An isolated DNA molecule according to claim 18, wherein the  
10 fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

19. An expression system transformed with a DNA molecule according to claim 10.

20. An expression system according to claim 19, wherein said  
15 DNA molecule is in proper sense orientation and correct reading frame.

21. A host cell transformed with a DNA molecule according to  
claim 10.

20

22. A host cell according to claim 21, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.

23. A host cell according to claim 21, wherein the DNA molecule  
25 is transformed with an expression system.

24. A transgenic plant transformed with the DNA molecule of  
claim 10.

25. A transgenic plant according to claim 24, wherein the plant is  
30 selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive,

- 64 -

cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

5

26. A transgenic plant according to claim 24, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

10

27. A transgenic plant seed transformed with the DNA molecule of claim 10.

15

28. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

20

29. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

25

30. A method of imparting disease resistance to plants comprising: applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to impart disease resistance.

30

31. A method according to claim 30, wherein plants are treated during said applying.

32. A method according to claim 30 wherein plant seeds are treated during said applying, said method further comprising:

5       planting the seeds treated with the fragment of the  
hypersensitive response elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.

33. A method of enhancing plant growth comprising:

10       applying a fragment of a hypersensitive response elicitor  
protein or polypeptide; which fragment does not elicit a hypersensitive response, in a  
non-infectious form to a plant or plant seed under conditions effective to enhance  
plant growth.

15       34. A method according to claim 33, wherein plants are treated  
during said applying.

35. A method according to claim 33, wherein plant seeds are treated during said applying, said method further comprising:

20       planting the seeds treated with the fragment of the  
hypersensitive response elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.

36. A method of insect control for plants comprising:

25       applying a fragment of a hypersensitive response elicitor protein or  
polypeptide, which fragment does not elicit a hypersensitive response, in a non-  
infectious form to a plant or plant seed under conditions effective to control insects.

37. A method according to claim 36, wherein plants are treated during said applying.

30

38. A method according to claim 36, wherein plant seeds are treated during said applying, said method further comprising:

- 66 -

planting the seeds treated with the fragment of the hypersensitive response elicitor in natural or artificial soil and propagating plants from the seeds planted in the soil.

- 5                    39.    A method of imparting disease resistance to plants comprising:  
                     providing a transgenic plant or plant seed transformed with a  
DNA molecule which encodes a fragment of a hypersensitive response elicitor protein  
or polypeptide, which fragment does not elicit a hypersensitive response, and  
                     growing the transgenic plant or transgenic plants produced  
10    from the transgenic plant seeds under conditions effective to impart disease resistance.
40.    A method according to claim 39, wherein a transgenic plant is  
provided.
- 15                   41.    A method according to claim 39, wherein a transgenic plant  
seed is provided.
42.    A method of enhancing plant growth comprising:  
                     providing a transgenic plant or a plant seed transformed with a  
20    DNA molecule which encodes a fragment of a hypersensitive response elicitor protein  
or polypeptide, which fragment does not elicit a hypersensitive response, and  
                     growing the transgenic plant or transgenic plants produced  
from the transgenic plant seeds under conditions effective to enhance plant growth.
- 25                   43.    A method according to claim 42, wherein a transgenic plant is  
provided.
44.    A method according to claim 42, wherein a transgenic plant  
seed is provided.
- 30                   45.    A method of insect control for plants comprising:

- 67 -

providing a transgenic plant or plant seed transformed with a DNA molecule which encodes a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, and growing the transgenic plant or transgenic plants produced from the transgenic plant seeds under conditions effective to control insects.

46. A method according to claim 45, wherein a transgenic plant is provided.

10 47. A method according to claim 45, wherein a transgenic plant seed is provided.

---

**THIS PAGE BLANK (USPTO)**

1/2

#1	HARPIN			
	1			403
#3		C-TERMINAL FRAGMENTS		
#4		105		403
#5			169	403
#6			210	403
#7			267	403
				343 403
#8	N-TERMINAL FRAGMENTS			
#9	1	75		
#10	1	104		
#11	1		168	
#12	1		266	
	1			342
#13	INTERNAL FRAGMENTS			
#14	76		209	
#15	76		168	
#16		105	209	
#17			169 209	
		105	168	
#18	SYNTHESIZED OLIGOPEPTIDES			
#19	99		209	
#20		137	204	150 179
#21		137	180	137 166
#22	105		180	121 150
#23		150	209	137 156
		150	180	

HARPIN FRAGMENTS DERIVED FROM HrpN OF ERWINIA AMYLOVORA

**FIG. 1****SUBSTITUTE SHEET (RULE 26)**

**THIS PAGE BLANK (USPTO)**



2/2

N1; 5' -GGGAATTCATATGAGTCTGAATACAAGTGGG-3'  
N76; 5' -GGGAATTCATATGGGCGGTGGCTTAGGCGGT-3'  
N99; 5' -GGCATATGTCGAACGCGCTGAACGATATG-3'  
N105; 5' -GGGAATTCATATGTTAGGCGGTTTCGCTGAAC-3'  
N110; 5' -GGCATATGCTGAACACGCTGGGCTCGAAA-3'  
N137; 5' -GGCATATGTCAACGTCCCAAACGACGAT-3'  
N150; 5' -GGCATATGTCCACCTCAGACTCCAGCG-3'  
N169; 5' -GGGAATTCATATGCAAAGCCTGTTTGGTGATGGG-3'  
N210; 5' -GGGAATTCATATGGGTAATGGTCTGAGCAAG-3'  
N267; 5' -GGGAATTCATATGAAAGCGGGCATTTCAGGCG-3'  
N343; 5' -GGGAATTCATATGACACCAGCCAGTATGGAGCAG-3'  
C75; 5' -GCAAGCTTAAACAGCCCACCGCCCATCAT-3'  
C104; 5' -GCAAGCTTAAATCGTTCAGCGCGTTCGACAG-3'  
C168; 5' -GCAAGCTTAAATATCTCGCTGAACATCTTCAGCAG-3'  
C180; 5' -GCAAGCTTAAAGGTGCCATCTTGCCCATCAC-3'  
C204; 5' -GCAAGCTTAAATCAGTGACTCCTTTTTTATAGGC-3'  
C209; 5' -GCAAGCTTAAACAGGCCCGACAGCGCATCAGT-3'  
C266; 5' -GCAAGCTTAAACCGATAACCGGTACCCACGGC-3'  
C342; 5' -GCAAGCTTAAATCCGTCGTCATCTGGCTTGCTCAG-3'  
C403; 5' -GCAAGCTTAAAGCCGCGCCCAGCTTG-3'

OLIGONUCLEOTIDE PRIMERS USED FOR THE CONSTRUCTION  
OF THE SUBCLONES OF ERWINIA AMYLOVORA HrpN

**FIG. 2**

**THIS PAGE BLANK (USPTO)**

## SEQUENCE LISTING

<110> Eden Bioscience Corp ration

<120> HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE  
ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

<130> 21829/32

<140>

<141>

<150> 60/103,050

<151> 1998-10-05

<160> 39

<170> PatentIn Ver. 2.0

<210> 1

<211> 31

<212> DNA

<213> Erwinia amylovora

<400> 1

gggaattcat atgagtctga atacaagtgg g

31

<210> 2

<211> 31

<212> DNA

<213> Erwinia amylovora

<400> 2

gggaattcat atgggcggtg gcttaggcgg t

31

<210> 3

<211> 29

<212> DNA

<213> Erwinia amylovora

<400> 3

ggcatatgtc gaacgcgctg aacgatatg

29

<210> 4

<211> 31

<212> DNA

<213> Erwinia amylovora

**THIS PAGE BLANK (USPTO)**

<400> 4  
gggaattcat atgtaggcg gttcgctgaa c

31

<210> 5  
<211> 29  
<212> DNA  
<213> *Erwinia amylovora*

<400> 5  
ggcatatgct gaacacgctg ggctcgaaa

29

<210> 6  
<211> 29  
<212> DNA  
<213> *Erwinia amylovora*

<400> 6  
ggcatatgct aacgtcccaa aacgacgat

29

<210> 7  
<211> 27  
<212> DNA  
<213> *Erwinia amylovora*

<400> 7  
ggcatatgct cacctcagac tccagcg

27

<210> 8  
<211> 34  
<212> DNA  
<213> *Erwinia amylovora*

<400> 8  
gggaattcat atgcaaagcc tggttggtga tggg

34

<210> 9  
<211> 31  
<212> DNA  
<213> *Erwinia amylovora*

<400> 9  
gggaattcat atgggtaatg gtctgagcaa g

31

<210> 10  
<211> 31  
<212> DNA  
<213> *Erwinia amylovora*

**THIS PAGE BLANK (USPTO)**

<400> 10  
gggaattcat atgaaagcgg gcattcaggc g 31

<210> 11  
<211> 34  
<212> DNA  
<213> Erwinia amylovora

<400> 11  
gggaattcat atgacaccag ccagtatgga gcag 34

<210> 12  
<211> 31  
<212> DNA  
<213> Erwinia amylovora

<400> 12  
gcaagcttaa cagcccacca ccgcccattca t 31

<210> 13  
<211> 31  
<212> DNA  
<213> Erwinia amylovora

<400> 13  
gcaagcttaa atcgttcagc gcgttcgaca g 31

<210> 14  
<211> 34  
<212> DNA  
<213> Erwinia amylovora

<400> 14  
gcaagcttaa tatctcgctg aacatcttca gcag 34

---

<210> 15  
<211> 30  
<212> DNA  
<213> Erwinia amylovora

<400> 15  
gcaagcttaa ggtgccatct tgcccatcac 30

<210> 16  
<211> 34  
<212> DNA  
<213> Erwinia amylovora

**THIS PAGE BLANK (USPTO)**



<400> 16  
gcaagcttaa atcagtgact ccttttttat aggc 34

<210> 17  
<211> 31  
<212> DNA  
<213> Erwinia amylovora

<400> 17  
gcaagcttaa caggcccgac agcgcatcag t 31

<210> 18  
<211> 31  
<212> DNA  
<213> Erwinia amylovora

<400> 18  
gcaagcttaa accgataccg gtacccacgg c 31

<210> 19  
<211> 34  
<212> DNA  
<213> Erwinia amylovora

<400> 19  
gcaagcttaa tccgtcgtca tctggcttgc tcag 34

<210> 20  
<211> 25  
<212> DNA  
<213> Erwinia amylovora

<400> 20  
gcaagcttaa gccgcgccca gcttg 25

<210> 21  
<211> 338  
<212> PRT  
<213> Erwinia chrysanthemi

<400> 21  
Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser  
1 5 10 15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser  
20 25 30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr

THIS PAGE BLANK (USPTO)

35	40	45
Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu		
50	55	60
Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser		
65	70	75
Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys		
85	90	95
Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp		
100	105	110
<del>Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln</del>		
<del>115</del>	<del>120</del>	<del>125</del>
Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met		
130	135	140
Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly		
145	150	155
Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly		
165	170	175
Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu		
180	185	190
Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala		
195	200	205
Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val		
210	215	220
Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp		
225	230	235
Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp		
245	250	255
Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys		
260	265	270
Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln		
275	280	285
Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr		

**THIS PAGE BLANK (USPTO**

290

295

300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
 305 310 315 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
 325 330 335

Asn Ala

&lt;210&gt; 22

&lt;211&gt; 2141

&lt;212&gt; DNA

<213> *Erwinia chrysanthemi*

&lt;400&gt; 22

cgatttttacc cgggtgaacg tgctatgacc gacagcatca cggatttcga caccggttacg 60  
 gcgtttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgcccga atccggcgctc 120  
 gatctgggtat ttcagtttgg ggacaccggg cgtgaactca tgatgcagat tcagccggggg 180  
 cagcaatata ccggcatgtt gcgcacgctg ctgcctcgtc gttatcagca ggcggcagag 240  
 tgcgatggct gccatctgtg cctgaacggc agcgatgtat tgatcctctg gtggccgctg 300  
 ccgtcggatc ccggcagtta tccgcagggtg atcgaacggt tgtttgaact ggcgggaatg 360  
 acgttgccgt cgctatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc 420  
 cgatcattaa gataaaggcg gcttttttta ttgcaaaacg gtaacgggtga ggaaccgttt 480  
 caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgctg 540  
 ggcataccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca 600  
 aattacgatc aaagcgacac tcggcgggtga tttgggcgctc tccgggtctgg ggctgggtgc 660  
 tcagggactg aaaggactga attccgcggc ttcacgctg gggtccagcg tggataaact 720  
 gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct 780  
 ggcgcagggg ctgggcgcca gctcgaaggg gctggggatg agcaatcaac tgggccagtc 840  
 tttcggcaat ggcgcgcagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcggcga 900  
 tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac 960  
 caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020  
 ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcactgtcgt ccattctcgg 1080  
 caacgggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggctt 1140  
 gcagggcctg agcggcgcggt gtgcattcaa ccagttgggt aatgccatcg gcattggcgt 1200  
 ggggcagaat gctgcgctga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260  
 ccgccacttt gtagataaag aagatcgcggt catggcgaaa gagatcggcc agtttatgga 1320  
 tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380  
 gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg 1440  
 cgccagcatg gacaaattcc gtcaggcgat gggatatgatc aaaagcgcggt tggcggggtga 1500  
 taccggcaat accaactga acctgcgtgg cgcgggcggt gcatcgctgg gtatcgatgc 1560  
 ggctgtcgtc ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620  
 atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680  
 ttattatgcg gtttatgcgg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740  
 acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttctc 1800

THIS PAGE BLANK (USPTO,

gtcgcgcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc 1860  
 cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gcccttttag 1920  
 cagatagatt gcggtttcgt aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980  
 gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040  
 aaaatagggc agtttttgcg tggatccgt ggggtgttcc ggctgacaa tcttgagttg 2100  
 gtctgcatc atctttctcc atctgggcga cctgatcggt t 2141

<210> 23

<211> 403

<212> PRT

<213> *Erwinia amylovora*

<400> 23

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
 1 5 10 15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
 20 25 30

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
 35 40 45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
 50 55 60

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
 65 70 75 80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
 85 90 95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
 100 105 110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
 115 120 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
 130 135 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
 145 150 155 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
 165 170 175

Gln Asp Gly Thr Gln Gly Ser Ser S r Gly Gly Lys Gln Pro Thr Glu  
 180 185 190

**THIS PAGE BLANK (USPTO)**



Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly  
 195 200 205  
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly  
 210 215 220  
 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu  
 225 230 235 240  
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln  
 245 250 255  
 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln  
 260 265 270  
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe  
 275 280 285  
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met  
 290 295 300  
 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro  
 305 310 315 320  
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser  
 325 330 335  
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn  
 340 345 350  
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
 355 360 365  
 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp  
 370 375 380  
 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu  
 385 390 395 400  
 Gly Ala Ala

&lt;210&gt; 24

&lt;211&gt; 1288

&lt;212&gt; DNA

&lt;213&gt; Erwinia amylovora

**THIS PAGE BLANK (USPTO)**

&lt;400&gt; 24

aagcttcggc atggcacggt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa 60  
 gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct 120  
 atcggcgggtg cgggcgga aaacgggttg ctgggtacca gtcgccagaa tgctgggttg 180  
 ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg 240  
 gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcgggtg tgggctgatg 300  
 ggcgggtggc taggcgggtg cttaggtaat ggcttgggtg gctcaggtgg cctgggcgaa 360  
 ggactgtcga acgcgctgaa cgatatgtta ggcgggttcgc tgaacacgct gggctcgaaa 420  
 ggcggcaaca ataccacttc aacaacaaat tccccgctgg accaggcgct ggggtattaac 480  
 tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac 540  
 ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggatgatggg 600  
 caagatggca cccagggcag ttctctctggg ggcaagcagc cgaccgaagg cgagcagaac 660  
 gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag 720  
 ctccctggca acgggggact gggaggtggg cagggcggta atgctggcac gggctctgac 780  
 ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccgggtgga ctaccagcag 840  
 ttaggtaacg ccgtgggtac cggtatcggt atgaaagcgg gcattcaggc gctgaatgat 900  
 atcggtagc acaggcacag ttcaaccctg tcttctcgtca ataaaggcga tcgggcgatg 960  
 gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac 1020  
 cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc 1080  
 aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc 1140  
 atgatcaaaa ggcccatggc gggtgatacc ggcaacggca acctgcaggc acgcgggtgcc 1200  
 ggtggttctt cgctgggtat tgatgccatg atggcgggtg atgccattaa caatatggca 1260  
 cttggcaagc tgggcgcggc ttaagctt 1288

&lt;210&gt; 25

&lt;211&gt; 1344

&lt;212&gt; DNA

<213> *Erwinia amylovora*

&lt;400&gt; 25

atgtcaattc ttacgcttaa caacaatacc tcgtcctcgc cgggtctgtt ccagtccggg 60  
 ggggacaacg ggcttgggtg tcataatgca aattctgcgt tggggcaaca acccatcgat 120  
 cggcaaacca ttgagcaaat ggctcaatta ttggcgggaa tggttaaagtc actgctatcg 180  
 ccacaatcag gtaatgcggc aaccggagcc ggtggcaatg accagactac aggagttggg 240  
 aacgctggcg gcctgaacgg acgaaaaggc acagcaggaa cactccgca gtctgacagt 300  
 cagaacatgc tgagttagat gggcaacaac gggctggatc aggccatcac gccgatggc 360  
 cagggcggcg ggcagatcgg cgataatcct ttactgaaag ccatgctgaa gcttattgca 420  
 cgcagatgg acggccaaag cgatcagttt ggccaacctg gtacgggcaa caacagtgcc 480  
 tcttccggta cttcttcacg tggcgggtcc ccttttaacg atctatcagg ggggaaggcc 540  
 ccttccggca actcccttc cggcaactac tctcccgta gtaccttctc acccccatcc 600  
 acgccaacgt cccctacctc accgcttgat ttcccttctt ctcccacaa agcagccggg 660  
 ggcagcacgc cggtaacgca tcacctgac cctgttggta gcgcgggcat cggggccgga 720  
 aattcgggtg ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc 780  
 gtgaaagcgg gtcaggtgtt tgatggcaaa ggacaaacct tcaccgccgg ttcagaatta 840  
 ggcgatggcg gccagtctga aaaccagaaa ccgctgttta tactggaaga cggtgccagc 900  
 ctgaaaaacg tcaccatggg cgacgacggg gcggatggta ttcattctta cggtgatgcc 960  
 aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac 1020

**THIS PAGE BLANK (USPTO)**

agcgcggggca aaaaatccca cgttgaaatc actaacagtt ccttcgagca cgcctctgac 1080  
 aagatcctgc agctgaatgc cgatactaac ctgagcgttg acaacgtgaa ggccaaagac 1140  
 tttggtactt ttgtacgcac taacggcggt caacagggtta actgggatct gaatctgagc 1200  
 catatcagcg cagaagacgg taagtctctc ttcgttaaaa gcgatagcga ggggctaaac 1260  
 gtcaatacca gtgatatctc actgggtgat gttgaaaacc actacaaagt gccgatgtcc 1320  
 gccaacctga aggtggctga atga 1344

<210> 26

<211> 447

<212> PRT

<213> *Erwinia amylovora*

<400> 26

Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu  
 1 5 10 15

Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser  
 20 25 30

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala  
 35 40 45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly  
 50 55 60

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly  
 65 70 75 80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro  
 85 90 95

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu  
 100 105 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp  
 115 120 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp  
 130 135 140

Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala  
 145 150 155 160

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser  
 165 170 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pr Ser Gly Asn Tyr Ser Pro  
 180 185 190

**THIS PAGE BLANK (USPTO)**

Val Ser Thr Phe Ser Pro Pro Ser Thr Pr Thr Ser Pr Thr Ser Pr  
 195 200 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro  
 210 215 220

Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly  
 225 230 235 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His  
 245 250 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln  
 260 265 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn  
 275 280 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val  
 290 295 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala  
 305 310 315 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr  
 325 330 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn  
 340 345 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp  
 355 360 365

---

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe  
 370 375 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser  
 385 390 395 400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser  
 405 410 415

Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu  
 420 425 430

Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu  
 435 440 445

**THIS PAGE BLANK (USPTO)**



&lt;210&gt; 27

&lt;211&gt; 5517

&lt;212&gt; DNA

<213> *Erwinia amylovora*

&lt;400&gt; 27

atggaattaa aatcactggg aactgaacac aaggcggcag tacacacagc ggcgcacaac 60  
 cctgtggggc atggtgttgc cttacagcag ggcagcagca gcagcagccc gcaaaatgcc 120  
 gctgcatcat tggcggcaga aggcaaaaat cgtgggaaaa tgccgagaat tcaccagcca 180  
 tctactgctg ctgatggtat cagcgtctgt caccagcaaa agaaatcctt cagtctcagg 240  
 ggctgttttg ggacgaaaaa attttccaga tcggcaccgc agggccagcc aggtaccacc 300  
 cacagcaaaag gggcaacatt gcgcgatctg ctggcgcggg acgacggcga aacgcagcat 360  
~~gaggcggccg cgccagatgc ggcggtttg acccgttcgg gggcggtcaa acgcccgaat 420~~  
 atggacgaca tggccggggc gccaatggtg aaaggtggca gcggcgaaga taaggtacca 480  
 acgcagcaaa aacggcatca gctgaacaat tttggccaga tgcgccaaac gatgttgagc 540  
 aaaatggctc acccggcttc agccaacgcc ggcgatcgcc tgcagcattc accgccgcac 600  
 atcccgggta gccaccacga aatcaaggaa gaaccgggtg gctccaccag caaggcaaca 660  
 acggcccacg cagacagagt ggaaatcgct caggaagatg acgacagcga attccagcaa 720  
 ctgcatcaac agcggctggc gcgcgaacgg gaaaatccac cgcagccgcc caaactcggc 780  
 gttgccacac cgattagcgc caggtttcag cccaaactga ctgcggttgc ggaaagcgct 840  
 cttgagggga cagataccac gcagtcaccc cttaagccgc aatcaatgct gaaaggaagt 900  
 ggagccgggg taacgccgct ggcggtaacg ctggataaag gcaagttgca gctggcaccg 960  
 gataatccac ccgcgctcaa tacgttggtg aagcagacat tgggtaaaga caccagcac 1020  
 tatctggcgc accatgccag cagcgacggt agccagcatc tgctgctgga caacaaaggc 1080  
 cacctgtttg atatcaaaag caccgccacc agctatagcg tgctgcacaa cagccacccc 1140  
 ggtgagataa agggcaagct ggcgcaggcg ggtactggct ccgtcagcgt agacggtaaa 1200  
 agcggcaaga tctcgtctgg gagcggtacg caaagtcaca aaaaaacaat gctaagccaa 1260  
 ccgggggaag cgcaccgttc cttattaacc ggcatttggc agcatcctgc tggcgccagc 1320  
 cggccgcagg gcgagtcgat ccgcctgcat gacgacaaaa ttcatactct gcatccggag 1380  
 ctgggcgtat ggcaatctgc ggataaagat acccacagcc agctgtctcg ccaggcagac 1440  
 ggtaagctct atgcgctgaa agacaaccgt accctgcaaa acctctccga taataaatcc 1500  
 tcagaaaagc tggctcgataa aatcaaatcg tattccggtg atcagcgggg gcaggtggcg 1560  
 atcctgacgg atactcccg ccgccataag atgagtatta tgccctcgct ggatgcttcc 1620  
 ccggagagcc atatttccct cagcctgcat tttgccgatg cccaccaggg gttattgcac 1680  
 gggaagtcgg agcttgaggc acaatctgtc gcgatcagcc atgggcgact ggttgtggcc 1740  
 gatagcgaag gcaagctggt tagcgccgcc attccgaagc aaggggatgg aaacgaactg 1800  
 aaaatgaaag ccatgcctca gcctgcgtc gatgaacatt ttggtcatga ccaccagatt 1860  
 tctggatttt tccatgacga ccacggccag cttaatgcgc tggtgaaaaa taacttcagg 1920  
 cagcagcatg cctgcccgtt gggtaacgat catcagtttc accccggctg gaacctgact 1980  
 gatgcgctgg ttatcgacaa tcagctgggg ctgcatcata ccaatcctga accgcatgag 2040  
 attcttgata tggggcattt aggcagcctg gcgttacagg agggcaagct tcactatttt 2100  
 gaccagctga ccaaaggggt gactggcgcg gagtcagatt gtaagcagct gaaaaaggc 2160  
 ctggatggag cagcttatct actgaaagac ggtgaagtga aacgcctgaa tattaatcag 2220  
 agcacctcct ctatcaagca cggaaacggaa aacgtttttt cgctgccgca tgtgcgcaat 2280  
 aaaccggagc cgggagatgc cctgcaaggg ctgaataaag acgataaggc ccaggccatg 2340  
 gcgggtgattt gggtaataaa atacctggcg ctgacggaaa aaggggacat tcgctccttc 2400

**THIS PAGE BLANK (USPTO)**

cagataaaac ccggcaccca gcagttggag cggccggcac aaactctcag ccgcgaaggt 2460  
 atcagcggcg aactgaaaga cattcatgtc gaccacaagc agaacctgta tgccttgacc 2520  
 caccagggag aggtgtttca tcagccgcgt gaagcctggc agaatgggtc cgaaagcagc 2580  
 agctggcaca aactggcggt gccacagagt gaaagtaagc taaaaagtct ggacatgagc 2640  
 catgagcaca aaccgattgc cacctttgaa gacggtagcc agcatcagct gaaggctggc 2700  
 ggctggcacg cctatgcggc acctgaacgc gggccgctgg cgggtgggtac cagcggttca 2760  
 caaaccgtct ttaaccgact aatgcagggg gtgaaaggca aggtgatccc aggcagcggg 2820  
 ttgacggtta agctctcggc tcagacgggg ggaatgaccg gcgccgaagg gcgcaaggtc 2880  
 agcagtaaat tttccgaaag gatccgcgcc tatgcgttca acccaacaat gtccacgcgc 2940  
 cgaccgatta aaaatgctgc ttatgccaca cagcacggct ggcagggggc tgagggggtg 3000  
 aagccgttgt acgagatgca gggagcgctg attaaacaac tggatgcgca taacgttcgt 3060  
 cataacgcgc cacagccaga tttgcagagc aaactggaaa ctctggattt aggcgaacat 3120  
 ggcgcagaat tgcttaacga catgaagcgc ttccgcgacg aactggagca gagtgcaccc 3180  
 cgctcggtga ccgttttagg tcaacatcag ggagtgtctaa aaagcaacgg tgaatatcaat 3240  
 agcgaattta agccatcgcc cggcaaggcg ttggtccaga gctttaacgt caatcgctct 3300  
 ggtcaggatc taagcaagtc actgcaacag gcagtacatg ccacgcgcgc atccgcagag 3360  
 agtaaaactgc aatccatgct ggggcacttt gtcagtgcgc ggggtggatat gagtcatcag 3420  
 aagggcgaga tcccgctggg ccgccagcgc gatccgaatg ataaaaccgc actgaccaa 3480  
 tcgcgtttta ttttagatac cgtgaccatc ggtgaactgc atgaactggc cgataaggcg 3540  
 aaactggtat ctgaccataa acccgatgcc gatcagataa aacagctgcg ccagcagttc 3600  
 gatacgctgc gtgaaaagcg gtatgagagc aatccggtga agcattacac cgatatgggc 3660  
 ttcaccata ataaggcgct ggaagcaaac tatgatgcgg tcaaagcctt tatcaatgcc 3720  
 ttttaagaaag agcaccacgg cgtcaatctg accacgcgta ccgtactgga atcacagggc 3780  
 agtgcgagc tggcgaaaga gctcaagaat acgctgttgt ccctggacag tggtgaaagt 3840  
 atgagcttca gccggtcata tggcgggggc gtcagcactg tctttgtgccc tacccttagc 3900  
 aagaaggtgc cagttccggt gatccccgga gccggcatca cgctggatcg cgcctataac 3960  
 ctgagcttca gtggtaccag cggcggtatg aacgtcagtt ttggccgcga cggcggggtg 4020  
 agtggttaaca tcatggctgc taccggccat gatgtgatgc cctatatgac cggtaagaaa 4080  
 accagtgcag gtaacgccag tgactgggtg agcgcaaac ataaaatcag cccggacttg 4140  
 cgatatggcg ctgctgtgag tggcaccctg caaggaacgc taaaaaacag cctgaagttt 4200  
 aagctgacag aggatgagct gcctggcttt atccatggct tgacgcagtg cacgttgacc 4260  
 ccggcagaac tgttgcaaaa ggggatcgaa catcagatga agcagggcag caaactgacg 4320  
 tttagcgtcg atacctcgcc aaatctggat ctgctgtccg gtatcaatct gaacgaagac 4380  
 ggcagtaaac caaatgggtg cactgcccgt gtttctgccc ggctaagtgc atcggcaaac 4440  
 ctggccgcgc gctcgcgtga acgcagcacc acctctggcc agtttggcag cagcacttcg 4500  
 gccagcaata accgcccaac ctccctcaac ggggtcggcg cgggtgtctaa cctgacggct 4560  
 gcttttagggg ttgccattc atctacgcat gaagggaac cggctcgggat cttcccgga 4620  
 tttacctga ccaatgtttc ggcagcgtg gcgctggata accgtacctc acagagtatc 4680  
 agcctggaat tgaagcgcgc ggagccggtg accagcaacg atatcagcga gttgacctc 4740  
 acgctgggaa aacactttta ggatagcgcc acaacgaaga tgcttgccgc tctcaaagag 4800  
 ttagatgacg ctaagcccgc tgaacaactg catattttac agcagcattt cagtgcacaa 4860  
 gatgtcgtcg gtgatgaacg ctacgaggcg gtgcgcaacc tgaaaaaact ggtgatcgt 4920  
 caacaggctg cggacagcca cagcatggaa ttaggatctg ccagtcacag cagcacctac 4980  
 aataatctgt cgagaataaa taatgacggc attgtcgagc tgctacacaa acatttcgat 5040  
 gcggcattac cagcaagcag tgccaaacgt cttgggtgaaa tgatgaataa cgatccggca 5100  
 ctgaaagata ttattaagca gctgcaaagt acgccgttca gcagcgccag cgtgtcgatg 5160  
 gagctgaaag atggtctgcg tgagcagacg gaaaaagcaa tactggacgg taaggctcgt 5220  
 cgtgaagaag tgggagtact tttccaggat cgtaacaact tgcgtgttaa atcggtcagc 5280

**THIS PAGE BLANK (USPTO)**

gtcagtcagt ccgtcagcaa aagcgaaggc ttcaataccc cagcgctggt actggggacg 5340  
 agcaacagcg ctgctatgag catggagcgc aacatcggaa ccattaattt taaatacggc 5400  
 caggatcaga acacccacg gcgatttacc ctggagggtg gaatagctca ggctaattccg 5460  
 caggtcgcat ctgcgcttac tgatttgaag aaggaagggc tggaaatgaa gagctaa 5517

<210> 28

<211> 1838

<212> PRT

<213> *Erwinia amylovora*

<400> 28

Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr  
 1 5 10 15

Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser  
 20 25 30

Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly  
 35 40 45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala  
 50 55 60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg  
 65 70 75 80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln  
 85 90 95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala  
 100 105 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala  
 115 120 125

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met  
 130 135 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro  
 145 150 155 160

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln  
 165 170 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp  
 180 185 190

Arg Leu Gln His Ser Pr Pro His Ile Pro Gly Ser His His Glu Ile

**THIS PAGE BLANK (USPTO)**

195	200	205
Lys Glu Glu Pr Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala		
210	215	220
Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln		
225	230	235 240
Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro		
	245	250 255
Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys		
	260	265 270
Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln		
	275	280 285
Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val		
	290	295 300
Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro		
	305	310 315 320
Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys		
	325	330 335
Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln		
	340	345 350
His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr		
	355	360 365
Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys		
	370	375 380
Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys		
	385	390 395 400
Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr		
	405	410 415
Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile		
	420	425 430
Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg		
	435	440 445
Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp		





450	455	460
Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp		
465	470	475 480
Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser		
485	490	495
Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser		
500	505	510
Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg		
515	520	525
<del>His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His</del>		
<del>530</del>	<del>535</del>	<del>540</del>
Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His		
545	550	555 560
Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg		
565	570	575
Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro		
580	585	590
Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His		
595	600	605
Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe		
610	615	620
His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg		
625	630	635 640
Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly		
645	650	655
Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His		
660	665	670
His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly		
675	680	685
Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr		
690	695	700
Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly		

**THIS PAGE BLANK (USPTO)**

705	710	715	720
Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu	725	730	735
Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val	740	745	750
Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu	755	760	765
Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly	770	775	780
Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe	785	790	795
Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu	805	810	815
Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His	820	825	830
Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln	835	840	845
Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys	850	855	860
Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser	865	870	875
His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln	885	890	895
Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro	900	905	910
Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met	915	920	925
Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys	930	935	940
Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val	945	950	955
Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr			

**THIS PAGE BLANK (USPTO)**

965	970	975
Met Ser Thr Pro Arg Pr	Ile Lys Asn Ala Ala Tyr Ala Thr Gln His	
980	985	990
Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly		
995	1000	1005
Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro		
1010	1015	1020
Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His		
1025	1030	1035
1040		
<del>Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu</del>		
<del>1045</del>	<del>1050</del>	<del>1055</del>
Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val		
1060	1065	1070
Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly		
1075	1080	1085
Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu		
1090	1095	1100
Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu		
1105	1110	1115
1120		
Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp		
1125	1130	1135
Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro		
1140	1145	1150
Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val		
1155	1160	1165
Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser		
1170	1175	1180
Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe		
1185	1190	1195
1200		
Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr		
1205	1210	1215
Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp		

**THIS PAGE BLANK (USPTO)**

1220	1225	1230
Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val		
1235	1240	1245
Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu		
1250	1255	1260
Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser		
1265	1270	1275 1280
Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val		
1285	1290	1295
<del>Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly</del>		
<del>1300</del>	<del>1305</del>	<del>1310</del>
Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly		
1315	1320	1325
Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile		
1330	1335	1340
Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys		
1345	1350	1355 1360
Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile		
1365	1370	1375
Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly		
1380	1385	1390
Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro		
1395	1400	1405

---

Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu		
1410	1415	1420
Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr		
1425	1430	1435 1440
Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn		
1445	1450	1455
Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser		
1460	1465	1470
Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg		

**THIS PAGE BLANK (USPTO)**



1475	1480	1485
Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn		
1490	1495	1500
Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala		
1505	1510	1515 1520
Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly		
1525	1530	1535
Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu		
1540	1545	1550
<del>Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu</del>		
<del>1555</del>	<del>1560</del>	<del>1565</del>
Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys		
1570	1575	1580
His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu		
1585	1590	1595 1600
Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His		
1605	1610	1615
Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg		
1620	1625	1630
Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser		
1635	1640	1645
Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser		
1650	1655	1660
Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp		
1665	1670	1675 1680
Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn		
1685	1690	1695
Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro		
1700	1705	1710
Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu		
1715	1720	1725
Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val		

**THIS PAGE BLANK (USPTO)**

1730                      1735                      1740  
 Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser  
 1745                      1750                      1755                      1760  
 Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu  
                          1765                      1770                      1775  
 Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile  
                          1780                      1785                      1790  
 Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg  
                          1795                      1800                      1805  
~~Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser~~  
                          1810                      1815                      1820  
 Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser  
 1825                      1830                      1835

<210> 29  
 <211> 420  
 <212> DNA  
 <213> *Erwinia amylovora*

<400> 29  
 atgacatcgt cacagcagcg gggtgaaagg tttttacagt atttctccgc cgggtgtaaa 60  
 acgcccatac atctgaaaga cggggtgtgc gccctgtata acgaacaaga tgaggaggcg 120  
 gcggtgctgg aagtaccgca acacagcgac agcctgttac tacactgccg aatcattgag 180  
 gctgaccac aaacttcaat aaccctgtat tcgatgctat tacagctgaa ttttgaaatg 240  
 gcggccatgc gcggctgttg gctggcgctg gatgaactgc acaacgtgcg tttatgtttt 300  
 cagcagtcgc tggagcatct ggatgaagca agtttttagcg atatcgttag cggttcac 360  
 gaacatgcgg cagaagtgcg tgagtatata gcgcaattag acgagagtag cgcggcataa 420

<210> 30  
 <211> 139  
 <212> PRT  
 <213> *Erwinia amylovora*

<400> 30  
 Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser  
     1                    5                    10                    15  
 Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu  
                     20                    25                    30  
 Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His

THIS PAGE BLANK (USPTO)

35	40	45
Ser Asp Ser Leu Leu Leu His Cys Arg Ile Il	Glu Ala Asp Pro Gln	
50	55	60
Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met		
65	70	75
Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val		
85	90	95
Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe		
100	105	110
<del>Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu</del>		
<del>115</del>	<del>120</del>	<del>125</del>
Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala		
130	135	

```
<210> 31
<211> 341
<212> PRT
<213> Pseudomonas syringae
```

```

<400> 31
Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
  1                      5                      10                      15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
      20                      25                      30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
  35                      40                      45

```

Arg	Asn	Gly	Gln	Leu	Asp	Asp	Ser	Ser	Pro	Leu	Gly	Lys	Leu	Leu	Ala		
50					55					60							
Lys	Ser	Met	Ala	Ala	Asp	Gly	Lys	Ala	Gly	Gly	Gly	Ile	Glu	Asp	Val		
65					70					75						80	
Ile	Ala	Ala	Leu	Asp	Lys	Leu	Ile	His	Glu	Lys	Leu	Gly	Asp	Asn	Phe		
85					90					95							
Gly	Ala	Ser	Ala	Asp	Ser	Ala	Ser	Gly	Thr	Gly	Gln	Gln	Asp	Leu	Met		
100					105					110							

**THIS PAGE BLANK (USPTO)**

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro M t  
130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
145 150 155 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
195 200 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser  
225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp  
245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val  
260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
305 310 315 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
325 330 335

Asn Gln Ala Ala Ala  
340

<210> 32

<211> 1026

**THIS PAGE BLANK (USPTO)**



&lt;212&gt; DNA

<213> *Pseudomonas syringae*

&lt;400&gt; 32

```

atgcagagtc tcagtcttaa cagcagctcg ctgcaaaccg cggcaatggc ccttgtcctg 60
gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagtgtgc 120
gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga 180
aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc 240
atcgctgcgc tggacaagct gatccatgaa aagctcgggtg acaacttcgg cgcgtctgcg 300
gacagcgcct cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc 360
aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac 420
gatatgccga tgctgaacaa gatcgcgcag ttcattggatg acaatcccgc acagtttccc 480
aagccggact cgggctcctg ggtgaacgaa ctcaagggaag acaacttcct tgatggcgac 540
gaaacggctg cgttccgttc ggcactcgac atcattggcc agcaactggg taatcagcag 600
agtgcgctg gcagtctggc agggacgggt ggagggtctg gcactccgag cagtttttcc 660
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccgggtc cggtgacagc 720
ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780
tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgagac cggtagctcg 840
gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggctt gctgctcaag 900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960
gcgcaaactc ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca 1020
gcctga                                     1026

```

&lt;210&gt; 33

&lt;211&gt; 1729

&lt;212&gt; DNA

<213> *Pseudomonas syringae*

&lt;400&gt; 33

```

tccacttcgc tgattttgaa attggcagat tcatagaaac gttcagggtg ggaaatcagg 60
ctgagtgcgc agatttcgtt gataagggtg tggtagctgg cattgttggt catttcaagg 120
cctctgagtg cgggtgcggag caataccagt cttcctgctg gcgtgtgcac actgagtcgc 180
aggcataggg atttcagttc cttgcgttgg ttgggcatat aaaaaagga acttttaaaa 240
acagtgcgat gagatgccgg caaaacggga accggctcgt gcgctttgcc actcacttcg 300
agcaagctca accccaaca tccacatccc tatcgaacgg acagcgatac ggccacttgc 360
tctggtaaac cctggagctg gcgtcgggtc aattgcccac ttagcgaggt aacgcagcat 420
gagcatcggc atcacacccc ggccgcaaca gaccaccacg ccaactcgatt ttccggcgct 480
aagcggcaag agtcctcaac caaacacgtt cggcgagcag aacactcagc aagcgatcga 540
cccagtgca ctgttggtcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga 600
cagcaccgtc cagaatccgc aggacgccag caagcccaac gacagccagt ccaacatcgc 660
taaattgatc agtgcatgta tcatgtcgtt gctgcagatg ctaccaact ccaataaaaa 720
gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcgggct 780
cggtagaccg tcggccgata gcggggcgcg cggtagaccg gatgagcagc gtggcgcgcg 840
cggtgatagc ccaagcgcaa caggcgggtg cggcgggtgat actccgaccg caacaggcgg 900
tggcggcagc ggtggcgcg gcacacccac tgcaacaggt ggcggcagcg gtggcacacc 960
cactgcaaca ggcggtggcg aggggtggcg aacaccgcaa atcactccgc agttggccaa 1020
ccctaaccgt acctcaggta ctggctcggt gtcggacacc gcaggttcta ccgagcaagc 1080
cggcaagatc aatgtggtga aagacaccat caaggctcggc gctggcgaag tctttgacgg 1140

```

**THIS PAGE BLANK (USPTO)**

ccacggcgca accttcactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca 1200  
 gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa 1260  
 cgaggtcgat ggcattccacg tgaagccaa aaacgctcag gaagtcacca ttgacaacgt 1320  
 gcatgcccag aacgtcgggtg aagacctgat tacggtcaaa ggcgagggag gcgcagcggt 1380  
 cactaatctg aacatcaaga acagcagtgc caaagggtgca gacgacaagg ttgtccagct 1440  
 caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggt 1500  
 tcgcaccaac ggtggcaagc agtttgatga catgagcatc gagctgaacg gcatcgaagc 1560  
 taaccacggc aagttcgccc tggtgaaaag cgacagtgac gatctgaagc tggcaacggg 1620  
 caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgacccaaca 1680  
 caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc 1729

&lt;210&gt; 34

&lt;211&gt; 424

&lt;212&gt; PRT

<213> Pseudomonas syringae

&lt;400&gt; 34

Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu  
 1 5 10 15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly  
 20 25 30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly  
 35 40 45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val  
 50 55 60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile  
 65 70 75 80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr  
 85 90 95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln  
 100 105 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser  
 115 120 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Asp Thr  
 130 135 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly  
 145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly

**THIS PAGE BLANK (USPTO)**

[illegible]

**THIS PAGE BLANK (USPTO)**

420

&lt;210&gt; 35

&lt;211&gt; 344

&lt;212&gt; PRT

<213> *Pseudomonas solanacearum*

&lt;220&gt;

<223> Description of Unknown Organism: *Pseudomonas solanacearum*

&lt;400&gt; 35

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln  
 1 5 10 15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser  
 20 25 30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile  
 35 40 45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly  
 50 55 60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala  
 65 70 75 80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser  
 85 90 95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met  
 100 105 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
 115 120 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
 130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
 145 150 155 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
 165 170 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
 180 185 190

**THIS PAGE BLANK (USPTO)**



Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
210 215 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
225 230 235 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
275 280 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
305 310 315 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
325 330 335

Gln Ser Thr Ser Thr Gln Pro Met  
340

<210> 36

<211> 1035

<212> DNA

<213> *Pseudomonas solanacearum*

<400> 36

atgtcagtcg gaaacatcca gagcccgctg aacctccccg gtctgcagaa cctgaacctc 60  
aacaccaaca ccaacagcca gcaatcgggc cagtcctgtc aagacctgat caagcaggtc 120  
gagaaggaca tcctcaacat catcgagccc ctctgtgcaga aggccgcaca gtcggcgggc 180  
ggcaacaccg gtaacaccgg caacgcgccc gccaaggacg gcaatgccaa cgcgggcggc 240  
aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc 300  
ggcaacgtcg acgacgcca caaccaggat ccgatgcaag cgctgatgca gctgctggaa 360  
gacctggtga agctgctgaa ggccggccctg cacatgcagc agcccggcgg caatgacaag 420  
ggcaacggcg tggcggtgc caacggcgcc aagggtgccg gcggccaggc cggcctggcc 480  
gaagcgctgc aggagatcga gcagatcctc gccagctcg gcggcgggcg tgctggcgcc 540  
ggcgcgcgcg gtggcggtgt cggcggtgct ggtggcgcg atggcggtc cggcggggt 600

**THIS PAGE BLANK (USPTO)**

```

ggcgccaggcg gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac 660
ggccccgcaga acgcaggcga tgtcaacggg gccaacggcg cggatgacgg cagcgaagac 720
cagggcgggc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctgggtgcag 780
atgatgcagc aaggcgggcct cggcgggcggc aaccaggcgc agggcggtc gaagggtgcc 840
ggcaacgcct cgccgggttc cggcgcggaac ccgggcgcgga accagcccgg ttcggcggtat 900
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc 960
gtccagatcc tgcagcagat gctggcgggcg cagaacggcg gcagccagca gtccacctcg 1020
acgcagccga tgtaa 1035

```

&lt;210&gt; 37

&lt;211&gt; 26

&lt;212&gt; PRT

<213> *Xanthomonas campestris* pv. *glycines*

&lt;400&gt; 37

```

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
  1             5             10             15

```

```

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
      20             25

```

&lt;210&gt; 38

&lt;211&gt; 20

&lt;212&gt; PRT

<213> *Xanthomonas campestris* pv. *pelargonii*

&lt;400&gt; 38

```

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
  1             5             10             15

```

```

Leu Leu Ala Met
      20

```

&lt;210&gt; 39

&lt;211&gt; 13

&lt;212&gt; PRT

<213> *Phytophthora megasperma*

&lt;400&gt; 39

```

Val Trp Asn Gln Pro Val Arg Gly Phe Lys Val Tyr Glu
  1             5             10

```

THIS PAGE BLANK (USPTO)

# INTERNATIONAL SEARCH REPORT

Int. Application No  
PCT/US 99/23181

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/195 C12N15/31 C12N1/21 C12N5/10 A01H5/00  
A01H5/10 C12N15/82

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NÜRNBERGER T, ET AL. : "High Affinity Binding of a Fungal Oligopeptide Elicitor to arslay Plasma Membranes Triggers Multiple Defense Responses" CELL, vol. 78, no. 3, 12 August 1994 (1994-08-12), pages 449-460, XP000882736 Cambridge, Mass. cited in the application the whole document	1,2,10, 11, 19-23, 30-32, 36-38
A	WO 98 32844 A (CORNELL RES FOUNDATION INC) 30 July 1998 (1998-07-30) the whole document	
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

6 March 2000

Date of mailing of the international search report

03/04/2000

Name and mailing address of the ISA

European Patent Office, P.B. 6818 Patentean 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Bilang, J

# INTERNATIONAL SEARCH REPORT

Inte Application No  
PCT/US 99/23181

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 24297 A (CORNELL RES FOUNDATION INC) 11 June 1998 (1998-06-11) the whole document	
A	WEI Z-M, ET AL.: "Harpin, an HR elicitor, activates both defense and growth systems in many commercially important crops" PHYTOPATHOLOGY, vol. 88, September 1998 (1998-09), page S96 XP000882741 abstract	
A	NIGGEMEYER J, ET AL.: "Characterization of the functional domains of harpin" PHYTOPATHOLOGY, vol. 88, September 1998 (1998-09), page S67 XP000882740 abstract	

# INTERNATIONAL SEARCH REPORT

In relation on patent family members

Index

Application No

PCT/US 99/23181

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
W0 9832844	A	30-07-1998	AU	6043198 A	18-08-1998
W0 9824297	A	11-06-1998	AU	5693598 A	29-06-1998
			EP	0957672 A	24-11-1999

**THIS PAGE BLANK (USPTO)**



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
1 November 2001 (01.11.2001)

PCT

(10) International Publication Number  
**WO 01/80639 A2**

(51) International Patent Classification<sup>7</sup>: A01N 37/46,  
63/00, 63/02

(21) International Application Number: PCT/US01/12468

(22) International Filing Date: 17 April 2001 (17.04.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/198,359 19 April 2000 (19.04.2000) US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant: EDEN BIOSCIENCE CORPORATION  
[US/US]; 11816 North Creek Parkway N., Bothell, WA  
98011-8205 (US).

Published:  
— without international search report and to be republished  
upon receipt of that report

(72) Inventors: WEI, Zhong-Min; 8250 125th Court, Kirkland, WA 98034 (US). QIU, Dewen; 126 NE 145th Street, Seattle, WA 98155 (US). REMICK, Dean; 110 NE Lakefront Court, Lake Placid, FL 33842 (US).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(74) Agents: GOLDMAN, Michael, L. et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 31051, Rochester, NY 14603-1051 (US).



WO 01/80639 A2

(54) Title: TREATMENT OF FRUITS OR VEGETABLES WITH HYPERSENSITIVE RESPONSE ELICITOR TO INHIBIT POSTHARVEST DISEASE OR DESICCATION

(57) Abstract: The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable, either by treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation, or by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant. Also disclosed are DNA constructs and expression systems, host cells, and transgenic plants containing the DNA construct.

**THIS PAGE BLANK (USPTO)**

## TREATMENT OF FRUITS OR VEGETABLES WITH HYPERSENSITIVE RESPONSE ELICITOR TO INHIBIT POSTHARVEST DISEASE OR DESICCATION

5                   This application claims benefit of U.S. Provisional Patent Application  
Serial No. 60/198,359, filed April 19, 2000, which is hereby incorporated by  
reference in its entirety.

### FIELD OF THE INVENTION

10

The present invention relates to methods of treating fruits or vegetables  
to inhibit postharvest diseases and/or desiccation of harvested fruits or vegetables.

### BACKGROUND OF THE INVENTION

15

Postharvest diseases are often extensions of disease occurring in the  
field or orchard. Brown rot of stone fruits (*Monilinia fructicola* (Wint.) Honey), for  
example, may cause blossom and twig blighting in the orchard. Infections in the  
orchard may not be visible at harvest if fruits are not refrigerated. *Colletotrichum*  
20 *gloeosporioides* (Penz.) Arx may attack blossoms or leaves and young fruit of citrus,  
avocados, mangos, papayas, and a wide range of other tropical and subtropical  
species; infections in developing fruit are usually latent, and rot lesions appear only at  
the onset of fruit ripening. *Pezicula malicorticis* (Jacks.) Nannfld. causes cankers of  
limbs of apples and pears; infections in developing fruit are latent, and active rotting  
25 usually commences only after the fruit has spent several months in storage and  
proceeds during -1°C storage because the organism is able to grow at very low  
temperatures. These fungi used as examples are able to penetrate the cuticle and  
epidermis of the fruit.

Whether capable of being penetrated directly or not, wounds are often  
30 the usual means by which the fungus enters fruit. Cuts, punctures, bruises, and  
abrasions cannot be avoided completely during harvest and handling. If the cuticle  
and epidermis are broken, spores find nutrients and humidity in fresh wounds ideal for  
spore germination and colonization. Separation of fruits from the parent plant at  
harvest creates an unavoidable wound that encourages stem-end rots.

Rots developing at the blossom end usually involve prior colonization of floral parts. For example, *Botrytis* blossom-end rot (*B. cinerea*) sometimes occurs in Bartlett pears after a month or two in storage at -1°C. Initiation of rot in fruit flesh is associated with old styles and stamens retained within the fruit. Floral infections occur in the senescing floral parts at the end of blossoming. Mostly these floral parts are invaded by *Alternaria* spp. and common saprophytic fungi, but *B. cinerea* also is found occasionally. Not all fruits having *B. cinerea*-invaded floral parts rot in storage, but a significant percentage do. By contrast, test fruits remain free from *Botrytis* blossom-end rot if the old floral parts of developing fruits are free from *B. cinerea*. Rotting of fruits in storage is greatly reduced by a single orchard spray with a fungicide at the end of blossoming.

Contact infection, by which mycelia grow from a rotting fruit to contact and penetrate nearby fruit, is an especially serious aspect of some very common postharvest pathogens. The ever-enlarging "nest" of rotting fruit tied together by fungus mycelia will involve all fruit in a container, if given sufficient time.

Disease or threat of disease dictates in large measure the manner in which perishable fruits are handled. In recent decades, fruits have been shipped to increasingly greater distances from points of production. Exploitation of these distant markets, however, may offer large economic benefits only if the life of the commodity is stretched to its limit. Diseases and disorders ordinarily manageable during handling and transcontinental transit and marketing may be excessive when transoceanic marine transport of longer duration is involved. Similarly, the extension of marketing periods by storing fruits until they near the end of their physiological life may cause additional disease problems. Losses are especially serious if they occur in market areas, because the costs of sorting, packaging, cooling, storage, and transportation, which may greatly exceed production costs, have already been incurred. Of even greater long-term importance may be an impaired reputation leading to reduced future sales.

Postharvest diseases of fruit cause 15 to 25% losses yearly in the fruit industry worldwide and much of this is due to rot caused by microorganisms. Fungicides, which have been the primary means of controlling postharvest diseases, have come under scrutiny as posing potential oncogenic risks when applied to

processed foods. Thus, research efforts have been intensified to develop biological control procedures for postharvest diseases of fruits and vegetables that pose less risk to human health and the environment.

5 Considerable attention has been placed on assessing the use of antagonistic microorganisms as a viable alternative to the use of synthetic fungicides. Two basic approaches are available for using antagonistic microorganisms to control postharvest diseases. Naturally occurring antagonists that already exist on fruit and vegetable surfaces have been shown to control several rot pathogens on diverse commodities. Alternatively, artificially introduced antagonists have been shown to be  
10 effective in biologically controlling postharvest pathogens.

Since 1983, an explosion of research has occurred in the area of biological control of postharvest diseases by artificially introduced antagonists, mostly on fruit diseases (Janisiewicz, "Biological Control of Diseases of Fruit," In Biocontrol of Plant Diseases II, Mukergie et al. (ed.), CRC Press, Boca Raton, pp.  
15 153-165 (1988) and Wilson et al., "Potential for Biological Control of Postharvest Plant Diseases," Plant Disease 69:375-378 (1985)). For example, rot on apples was controlled with yeast (Wisniewski et al., "Biological Control of Postharvest Diseases of Fruit: Inhibition of *Botrytis* Rot on Apples by an Antagonistic Yeast," Proc. Electron Microsc. Soc. Am. 46:290-91 (1988)), while brown rot in apricots was  
20 controlled with *Bacillus subtilis* (Pusey et al., "Postharvest Biological Control of Stone Fruit Brown Rot by *Bacillus subtilis*," Plant Dis. 68:753-56 (1984)). Mold incidence was reduced from 35% to 8% in lemon peel by a species of *Trichoderma* (De Matos, "Chemical and Microbiological Factors Influencing the Infection of Lemons by *Geotrichum candidum* and *Penicillium digitatum*," Ph.D. dissertation,  
25 University of California, Riverside, 106 pp. (1983)). Biocontrol of citrus rot pathogens was demonstrated with *Bacillus subtilis* (Singh et al., "*Bacillus subtilis* as a Control Agent Against Fungal Pathogens of Citrus Fruit," Trans. Br. Mycol. Soc. 83:487-90 (1984)). Such antagonists have various modes of action: antibiosis or competition for nutrients and space or both, induction of resistance in the host tissue,  
30 and direct interaction with the pathogen (Wilson et al., "Biological Control of Postharvest Diseases of Fruits and Vegetables: An Emerging Technology," Annu. Rev. Phytopathol. 27:425-441 (1989)).

While treatment with antagonistic bacterial or fungal species may be, at least to some extent, effective in controlling postharvest diseases, there are a number of factors which must be considered before this approach is used in commercial applications. First, the antagonists must be grown and maintained for use in treatments. This may result in significant expense and regulatory burdens depending on when and how frequently such antagonists would be applied. Also, it is questionable whether growers would want to maintain bioreactors for growing and propagating particular antagonist strains. Second, the efficacy of those antagonists may depend on storage conditions during shipment of harvested fruit. Some antagonists may not be able to tolerate variations in conditions during shipment, thereby allowing the pathogens to overcome any inhibitory effects of the antagonists. Given the above problems, it is not surprising that few of the antagonists reported to control plant pathogens have been successfully transferred from the laboratory into the field or postharvest environment.

Thus, there still exists a need to provide an effective, commercially viable method for treating fruits and vegetables to control postharvest diseases which avoids entirely or otherwise significantly reduces the need for fungicide treatments. In particular, it would be desirable to provide an effective, practicable treatment which presents little or no harm to humans or the environment.

The present invention is directed to overcoming these and other deficiencies in the art.

## SUMMARY OF THE INVENTION

The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

Another aspect of the present invention relates to a DNA construct that includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule, the promoter being effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule, wherein expression of the DNA molecule in fruit or vegetable tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation. Also disclosed are expression systems, host cells, and transgenic plants which contain a heterologous DNA construct of the present invention.

By the present invention, the hypersensitive response elicitor protein or polypeptide can be used to inhibit or otherwise control postharvest diseases (i.e., caused by pathogens) in fruits or vegetables. Likewise, such treatment can also inhibit postharvest desiccation of treated fruits or vegetables. In achieving these objectives, the present invention enables produce growers, warehouse packers, shippers, and suppliers to process, handle, and store fruits and vegetables with reduced losses caused by postharvest disease and desiccation. As a result, the cost of bringing fruits and vegetables from the field to the consumer can be reduced. Importantly, the quality of the treated fruits or vegetables is improved.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

For use in accordance with these methods, suitable hypersensitive response elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

Exemplary hypersensitive response elicitor proteins and polypeptides from bacterial sources include, without limitation, the hypersensitive response elicitors derived from *Erwinia* species (e.g., *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, etc.), *Pseudomonas* species (e.g., *Pseudomonas syringae*, *Pseudomonas solanacearum*, etc.), and *Xanthomonas* species (e.g., *Xanthomonas campestris*). In addition to hypersensitive response elicitors from these Gram-negative bacteria, it is possible to use elicitors derived from Gram-positive bacteria. One example is the hypersensitive response elicitor derived from *Clavibacter michiganensis* subsp. *sepedonicus*.

Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors (i.e., elicitors) from various *Phytophthora* species (e.g., *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, *Phytophthora citrophthora*, etc.).

Preferably, the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*, *Erwinia amylovora*, *Pseudomonas syringae*, or *Pseudomonas solanacearum*.

A hypersensitive response elicitor protein or polypeptide from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser
1				5					10					15	
Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser
			20					25					30		
Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr
		35					40					45			
Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu
	50					55					60				
Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser
65					70					75				80	



5 Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys  
     85 90 95  
 Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp  
     100 105 110  
 10 Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln  
     115 120 125  
 Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met  
     130 135 140  
 Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly  
     145 150 155 160  
 Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly  
     165 170 175  
 15 Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu  
     180 185 190  
 Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala  
     195 200 205  
 Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val  
     210 215 220  
 20 Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp  
     225 230 235 240  
 Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp  
     245 250 255  
 Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys  
     260 265 270  
 25 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln  
     275 280 285  
 Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr  
     290 295 300  
 30 Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
     305 310 315 320  
 Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
     325 330 335  
 Asn Ala

35 This hypersensitive response elicitor protein or polypeptide has a molecular weight of  
 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains  
 substantially no cysteine. This *Erwinia chrysanthemi* hypersensitive response elicitor  
 protein or polypeptide is encoded by a DNA molecule having a nucleotide sequence  
 corresponding to SEQ. ID. No. 2 as follows:

40

cgattttacc cgggtgaacg tgctatgacc gacagcatca cgggtattcga caccggttacg 60  
 gcgttttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgccgca atccggcgctc 120  
 gatctggtat ttcagtttgg ggacaccggg cgtgaactca tgatgcagat tcagccggggg 180  
 cagcaatatc ccggcatgtt gcgcacgctg ctcgctcgctc gttatcagca ggcggcgagag 240

5      tgcgatggct gccatctgtg cctgaacggc agcgatgtat tgatcctctg gtggccgctg 300  
        ccgtcggatc ccggcagtta tccgcagggtg atcgaacggt tgtttgaact ggcggaatg 360  
        acgttgccgt cgctatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc 420  
        cgatcattaa gataaaggcg gcttttttta ttgcaaaacg gtaacgggtga ggaaccgttt 480  
        caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgtg 540  
        ggcatccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca 600  
        aattacgata aaagcgcaca tcggcgggtga tttgggcgtc tccgggtctgg ggctgggtgc 660  
        tcagggactg aaaggactga attccgcggc ttcacgtctg ggttccagcg tggataaact 720  
        gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct 780  
 10      ggcgccagggg ctgggcgccca gctcgaaggg gctggggatg agcaatcaac tgggccagtc 840  
        tttcggcaat ggcgcgagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcgccga 900  
        tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac 960  
        caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020  
        ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcactgtcgt ccattctcgg 1080  
 15      caacggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggctt 1140  
        gcagggcctg agcggcgcg gtgcattcaa ccagttgggt aatgccatcg gcatgggcgt 1200  
        ggggcagaat gctgcgtga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260  
        ccgccacttt gtagataaag aagatcgcg catggcgaaa gagatcggcc agtttatgga 1320  
        tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380  
 20      gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg 1440  
        cgccagcatg gacaaattcc gtcaggcgat gggatgatc aaaagcgcg tggcggtga 1500  
        taccggcaat accaacctga acctgcgtgg cgcgggcggt gcatoctggt gtatcgatgc 1560  
        ggctgtcgtc ggcgataaaa tagccaacat gtcgtgggt aagctggcca acgcctgata 1620  
        atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680  
 25      ttattatgcg gtttatgcg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740  
        acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttctc 1800  
        gtcgctcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc 1860  
        cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gcccttttag 1920  
        cagatagatt gcggtttcgt aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980  
 30      gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040  
        aaaatagggc agtttttgcg tggatatcgt ggggtgttcc ggcctgacaa tcttgagttg 2100  
        gttcgtcacc atctttctcc atctgggcga cctgatcggt t 2141

35      The above nucleotide and amino acid sequences are disclosed and further described in  
        U.S. Patent No. 5,850,015 to Bauer et al. and U.S. Patent No. 5,776,889 to Wei et al.,  
        which are hereby incorporated by reference in their entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

5	Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser	1 5 10 15
	Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln	20 25 30
10	Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn	35 40 45
	Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met	50 55 60
	Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu	65 70 75 80
15	Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu	85 90 95
	Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr	100 105 110
20	Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro	115 120 125
	Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser	130 135 140
	Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln	145 150 155 160
25	Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly	165 170 175
	Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu	180 185 190
30	Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly	195 200 205
	Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly	210 215 220
	Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu	225 230 235 240
35	Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln	245 250 255
	Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln	260 265 270
40	Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe	275 280 285
	Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met	290 295 300
	Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro	305 310 315 320
45	Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser	325 330 335

[illegible]

This hypersensitive response elicitor protein or polypeptide has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor protein or polypeptide has substantially no cysteine. The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

20	aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa	60
	gaggaatacgt ttatgagttct gaatacaagt gggctgggag cgtcaacgat gcaaattttct	120
	atcggcggtg cgggcggaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg	180
	ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg	240
25	gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcgggtg tgggctgatg	300
	ggcggtggtc taggcgggtg cttaggtaat ggcttgggtg gctcaggtgg cctgggcgaa	360
	ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa	420
	ggcggcaaca ataccacttc aacaacaaat tcccgcgtgg accaggcgct gggattatac	480
	tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac	540
30	ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggatgatggg	600
	caagatggca cccagggcag ttccctctggg ggcaagcagc cgaccgaagg cgagcagaac	660
	gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaattg tctgagccag	720
	ctccttggca acgggggact gggagggtgg cagggcggtg atgctggcac gggctctgac	780
	ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccggtgga ctaccagcag	840
35	ttaggtaacg ccgtgggtac cggtatcggg atgaaagcgg gcattcaggc gctgaatgat	900
	atcgggtacgc acaggcacag ttcaaccggt tctttcgtca ataaaggcga tcgggcgatg	960
	gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac	1020
	cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc	1080
	aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc	1140

```

atgatcaaaa ggcccatggc ggggtgatacc ggcaacggca acctgcaggc acgcggtgcc 1200
gggtgggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca 1260
cttggaagc tgggcgcggc ttaagctt 1288

```

5 The above nucleotide and amino acid sequences are disclosed are further described in U.S. Patent No. 5,849,868 to Beer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No.

10 5 as follows:

	Met	Ser	Ile	Leu	Thr	Leu	Asn	Asn	Asn	Thr	Ser	Ser	Ser	Pro	Gly	Leu	
	1				5					10					15		
	Phe	Gln	Ser	Gly	Gly	Asp	Asn	Gly	Leu	Gly	Gly	His	Asn	Ala	Asn	Ser	
15				20					25					30			
	Ala	Leu	Gly	Gln	Gln	Pro	Ile	Asp	Arg	Gln	Thr	Ile	Glu	Gln	Met	Ala	
			35					40					45				
	Gln	Leu	Leu	Ala	Glu	Leu	Leu	Lys	Ser	Leu	Leu	Ser	Pro	Gln	Ser	Gly	
		50					55					60					
20	Asn	Ala	Ala	Thr	Gly	Ala	Gly	Gly	Asn	Asp	Gln	Thr	Thr	Gly	Val	Gly	
	65				70						75				80		
	Asn	Ala	Gly	Gly	Leu	Asn	Gly	Arg	Lys	Gly	Thr	Ala	Gly	Thr	Thr	Pro	
				85						90					95		
25	Gln	Ser	Asp	Ser	Gln	Asn	Met	Leu	Ser	Glu	Met	Gly	Asn	Asn	Gly	Leu	
				100					105					110			
	Asp	Gln	Ala	Ile	Thr	Pro	Asp	Gly	Gln	Gly	Gly	Gly	Gln	Ile	Gly	Asp	
			115					120					125				
	Asn	Pro	Leu	Leu	Lys	Ala	Met	Leu	Lys	Leu	Ile	Ala	Arg	Met	Met	Asp	
		130					135					140					
30	Gly	Gln	Ser	Asp	Gln	Phe	Gly	Gln	Pro	Gly	Thr	Gly	Asn	Asn	Ser	Ala	
	145				150						155					160	
	Ser	Ser	Gly	Thr	Ser	Ser	Gly	Gly	Ser	Pro	Phe	Asn	Asp	Leu	Ser		
				165					170					175			
35	Gly	Gly	Lys	Ala	Pro	Ser	Gly	Asn	Ser	Pro	Ser	Gly	Asn	Tyr	Ser	Pro	
				180				185						190			
	Val	Ser	Thr	Phe	Ser	Pro	Pro	Ser	Thr	Pro	Thr	Ser	Pro	Thr	Ser	Pro	
			195					200					205				
	Leu	Asp	Phe	Pro	Ser	Ser	Pro	Thr	Lys	Ala	Ala	Gly	Gly	Ser	Thr	Pro	
		210					215					220					
40	Val	Thr	Asp	His	Pro	Asp	Pro	Val	Gly	Ser	Ala	Gly	Ile	Gly	Ala	Gly	
	225				230						235					240	
	Asn	Ser	Val	Ala	Phe	Thr	Ser	Ala	Gly	Ala	Asn	Gln	Thr	Val	Leu	His	
				245						250					255		

	Asp	Thr	Ile	Thr	Val	Lys	Ala	Gly	Gln	Val	Phe	Asp	Gly	Lys	Gly	Gln	
				260					265					270			
	Thr	Phe	Thr	Ala	Gly	Ser	Glu	Leu	Gly	Asp	Gly	Gly	Gln	Ser	Glu	Asn	
			275					280					285				
5	Gln	Lys	Pro	Leu	Phe	Ile	Leu	Glu	Asp	Gly	Ala	Ser	Leu	Lys	Asn	Val	
		290					295					300					
	Thr	Met	Gly	Asp	Asp	Gly	Ala	Asp	Gly	Ile	His	Leu	Tyr	Gly	Asp	Ala	
	305					310					315					320	
	Lys	Ile	Asp	Asn	Leu	His	Val	Thr	Asn	Val	Gly	Glu	Asp	Ala	Ile	Thr	
10				325					330						335		
	Val	Lys	Pro	Asn	Ser	Ala	Gly	Lys	Lys	Ser	His	Val	Glu	Ile	Thr	Asn	
			340					345						350			
	Ser	Ser	Phe	Glu	His	Ala	Ser	Asp	Lys	Ile	Leu	Gln	Leu	Asn	Ala	Asp	
			355					360					365				
15	Thr	Asn	Leu	Ser	Val	Asp	Asn	Val	Lys	Ala	Lys	Asp	Phe	Gly	Thr	Phe	
	370					375						380					
	Val	Arg	Thr	Asn	Gly	Gly	Gln	Gln	Gly	Asn	Trp	Asp	Leu	Asn	Leu	Ser	
	385				390						395					400	
	His	Ile	Ser	Ala	Glu	Asp	Gly	Lys	Phe	Ser	Phe	Val	Lys	Ser	Asp	Ser	
20				405					410						415		
	Glu	Gly	Leu	Asn	Val	Asn	Thr	Ser	Asp	Ile	Ser	Leu	Gly	Asp	Val	Glu	
			420					425					430				
	Asn	His	Tyr	Lys	Val	Pro	Met	Ser	Ala	Asn	Leu	Lys	Val	Ala	Glu		
25			435				440					445					

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

	atgtcaattc	ttacgcttaa	caacaatacc	tcgtcctcgc	cgggtctggt	ccagtccggg	60
	ggggacaacg	ggcttggtgg	tcataatgca	aattctgcgt	tggggcaaca	accatcgat	120
35	cggcaaacca	ttgagcaaat	ggctcaatta	ttggcggaac	tgttaaagtc	actgctatcg	180
	ccacaatcag	gtaatgcggc	aaccggagcc	ggtggcaatg	accagactac	aggagttggt	240
	aacgctggcg	gcctgaacgg	acgaaaaggc	acagcaggaa	ccactccgca	gtctgacagt	300
	cagaacatgc	tgagtgagat	gggcaacaac	gggctggatc	aggccatcac	gcccgatggc	360
	cagggcggcg	ggcagatcgg	cgataatcct	ttactgaaag	ccatgctgaa	gcttattgca	420
40	cgcatgatgg	acggccaaag	cgatcagttt	ggccaacctg	gtacgggcaa	caacagtgcc	480
	tcttccggta	cttcttcata	tggcggttcc	ccttttaacg	atctatcagg	ggggaaggcc	540
	ccttccggca	actccccttc	cggcaactac	tctcccgta	gtaccttctc	acccccatcc	600
	acgccaacgt	cccctacctc	accgcttgat	ttcccttctt	ctccaccaa	agcagccggg	660

```

ggcagcacgc cggttaaccga tcatcctgac cctggttgga gcgcgggcat cggggccgga 720
aattcggtag ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc 780
gtgaaagcgg gtcaggtgtt tgatggcaaa ggacaaacct tcaccgccgg ttcagaatta 840
ggcgaatggcg gccagtctga aaaccagaaa ccgctgttta tactggaaga cggtgccagc 900
5 ctgaaaaacg tcaccatggg cgacgacggg gcggatggta ttcattctta cggatgatgcc 960
aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac 1020
agcgcggggca aaaaatccca cgttgaaatc actaacagtt ccttcgagca cgcctctgac 1080
aagatcctgc agctgaatgc cgatactaac ctgagcgttg acaacgtgaa ggccaaagac 1140
tttggtactt ttgtacgcac taacggcggg caacagggtg actgggatct gaatctgagc 1200
10 catatcagcg cagaagacgg taagttctcg ttcgttaaaa gcgatagcga ggggctaaac 1260
gtcaatacca gtgatatctc actgggtgat gttgaaaacc actacaaagt gccgatgtcc 1320
gccaacctga aggtgggtga-atga 1344

```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent Application Serial No. 09/120,927 to Beer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

```

20 Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
    1          5          10          15
Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
    20          25          30
25 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
    35          40          45
Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
    50          55          60
30 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
    65          70          75          80
Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
    85          90          95
Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
    100         105         110
35 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
    115         120         125
Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
    130         135         140
40 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
    145         150         155         160
Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
    165         170         175

```

	Leu	Asp	Gly	Asp	Glu	Thr	Ala	Ala	Phe	Arg	Ser	Ala	Leu	Asp	Ile	Ile	
				180					185					190			
	Gly	Gln	Gln	Leu	Gly	Asn	Gln	Gln	Ser	Asp	Ala	Gly	Ser	Leu	Ala	Gly	
			195					200					205				
5	Thr	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ser	Phe	Ser	Asn	Asn	Ser	Ser	
		210					215						220				
	Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser	
	225					230					235					240	
10	Gly	Asn	Thr	Arg	Gly	Glu	Ala	Gly	Gln	Leu	Ile	Gly	Glu	Leu	Ile	Asp	
					245					250					255		
	Arg	Gly	Leu	Gln	Ser	Val	Leu	Ala	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Val	
				260					265						270		
	Asn	Thr	Pro	Gln	Thr	Gly	Thr	Ser	Ala	Asn	Gly	Gly	Gln	Ser	Ala	Gln	
			275					280					285				
15	Asp	Leu	Asp	Gln	Leu	Leu	Gly	Gly	Leu	Leu	Leu	Lys	Gly	Leu	Glu	Ala	
	290						295					300					
	Thr	Leu	Lys	Asp	Ala	Gly	Gln	Thr	Gly	Thr	Asp	Val	Gln	Ser	Ser	Ala	
	305					310					315					320	
20	Ala	Gln	Ile	Ala	Thr	Leu	Leu	Val	Ser	Thr	Leu	Leu	Gln	Gly	Thr	Arg	
					325					330						335	
	Asn	Gln	Ala	Ala	Ala												
					340												

25 This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., et al., "*Pseudomonas syringae* pv. *syringae* Harpin<sub>PS</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby

30 incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 8 as follows:

	atgcagagtc	tcagttctaa	cagcagctcg	ctgcaaacc	cggcaatggc	ccttgctctg	60
35	gtacgtcctg	aagccgagac	gactggcagt	acgtcgagca	aggcgcttca	ggaagttgtc	120
	gtgaagctgg	ccgaggaact	gatgcgcaat	ggtcaactcg	acgacagctc	gccattggga	180
	aaactgttgg	ccaagtcgat	ggccgcagat	ggcaaggcgg	gcggcggtat	tgaggatgtc	240
	atcgctgcgc	tggacaagct	gatccatgaa	aagctcgggt	acaacttcgg	cgcgtctgcg	300
	gacagcgcct	cgggtaccgg	acagcaggac	ctgatgactc	aggtgctcaa	tggcctggcc	360
40	aagtcgatgc	tcgatgatct	tctgaccaag	caggatggcg	ggacaagctt	ctccgaagac	420
	gatatgccga	tgctgaacaa	gatcgcgag	ttcatggatg	acaatcccgc	acagtttccc	480



```

aagccggact cgggctcctg ggtgaacgaa ctcaaggaag acaacttcct tgatggcgac 540
gaaacggctg cgttccgttc ggcaactcgac atcattggcc agcaactggg taatcagcag 600
agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcaactccgag cagtttttcc 660
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccgggtcc cggtgacagc 720
5 ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780
tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgcagac cggtagctcg 840
gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggctt gctgctcaag 900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960
gcgcaaatcg ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca 1020
10 gcctga 1026

```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,708,139 to Collmer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

15 Another hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

```

20 Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
   1           5           10           15
   Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
           20           25           30
   Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
           35           40           45
25 Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
   50           55           60
   Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
   65           70           75           80
30 Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
           85           90           95
   Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
           100          105          110
   Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
           115          120          125
35 Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
   130          135          140
   Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
   145          150          155          160
40 Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
           165          170          175
   Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
           180          185          190

```

	Pro	Gln	Ile	Thr	Pro	Gln	Leu	Ala	Asn	Pro	Asn	Arg	Thr	Ser	Gly	Thr	
			195					200					205				
	Gly	Ser	Val	Ser	Asp	Thr	Ala	Gly	Ser	Thr	Glu	Gln	Ala	Gly	Lys	Ile	
		210					215					220					
5	Asn	Val	Val	Lys	Asp	Thr	Ile	Lys	Val	Gly	Ala	Gly	Glu	Val	Phe	Asp	
	225				230					235						240	
	Gly	His	Gly	Ala	Thr	Phe	Thr	Ala	Asp	Lys	Ser	Met	Gly	Asn	Gly	Asp	
					245					250					255		
10	Gln	Gly	Glu	Asn	Gln	Lys	Pro	Met	Phe	Glu	Leu	Ala	Glu	Gly	Ala	Thr	
				260					265					270			
	Leu	Lys	Asn	Val	Asn	Leu	Gly	Glu	Asn	Glu	Val	Asp	Gly	Ile	His	Val	
			275					280					285				
	Lys	Ala	Lys	Asn	Ala	Gln	Glu	Val	Thr	Ile	Asp	Asn	Val	His	Ala	Gln	
		290					295					300					
15	Asn	Val	Gly	Glu	Asp	Leu	Ile	Thr	Val	Lys	Gly	Glu	Gly	Gly	Ala	Ala	
	305					310					315					320	
	Val	Thr	Asn	Leu	Asn	Ile	Lys	Asn	Ser	Ser	Ala	Lys	Gly	Ala	Asp	Asp	
					325					330					335		
20	Lys	Val	Val	Gln	Leu	Asn	Ala	Asn	Thr	His	Leu	Lys	Ile	Asp	Asn	Phe	
				340					345					350			
	Lys	Ala	Asp	Asp	Phe	Gly	Thr	Met	Val	Arg	Thr	Asn	Gly	Gly	Lys	Gln	
			355					360					365				
	Phe	Asp	Asp	Met	Ser	Ile	Glu	Leu	Asn	Gly	Ile	Glu	Ala	Asn	His	Gly	
		370					375					380					
25	Lys	Phe	Ala	Leu	Val	Lys	Ser	Asp	Ser	Asp	Asp	Leu	Lys	Leu	Ala	Thr	
	385					390					395					400	
	Gly	Asn	Ile	Ala	Met	Thr	Asp	Val	Lys	His	Ala	Tyr	Asp	Lys	Thr	Gln	
					405					410					415		
30	Ala	Ser	Thr	Gln	His	Thr	Glu	Leu									
				420													

This protein or polypeptide is acidic, glycine-rich, lacks cysteine, and is deficient in aromatic amino acids. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ.

35 ID. No. 10 as follows:

	tccacttcgc	tgattttgaa	attggcagat	tcatagaaac	gttcaggtgt	ggaaatcagg	60
	ctgagtgcgc	agatttcggt	gataaggggtg	tggtactggt	cattgtttggt	catttcaagg	120
	cctctgagtg	cgggtcggag	caataccagt	cttcctgctg	gcgtgtgcac	actgagtcgc	180
40	agggcataggc	atttcagttc	cttgcgttgg	ttgggcatat	aaaaaaagga	acttttaaaa	240
	acagtgcatt	gagatgccg	caaaacggga	accggtcgct	gcgctttgcc	actcacttcg	300
	agcaagctca	accccaaaca	tccacatccc	tatcgaacgg	acagcgatac	ggccacttgc	360
	tctggtaaac	cctggagctg	gcgtcggtcc	aattgcccac	ttagcgaggt	aacgcagcat	420
	gagcatcggc	atcacacccc	ggccgcaaca	gaccaccacg	ccactcgatt	tttcggcgct	480

```

aagcggcaag agtcctcaac caaacacggt cggcgagcag aacactcagc aagcgatcga 540
cccgagtgcg ctgttggtcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga 600
cagcacccgc cagaatccgc aggacgccag caagcccaac gacagccagt ccaacatcgc 660
taaattgata agtgcattga tcatgtcggt gctgcagatg ctcaccaact ccaataaaaa 720
5 gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcggggt 780
cggtacaccg tcggccgata gcggggggcg cggtacaccg gatgcgacag gtggcggcgg 840
cggtgatacg ccaagcgcaa caggcgggtg cggcgggtgat actccgaccg caacaggcgg 900
tggcggcagc ggtggcggcg gcacaccac tgcaacaggt ggcggcagcg gtggcacacc 960
cactgcaaca ggcggtggcg aggggtggcg aacaccgcaa atcactccgc agttggccaa 1020
10 ccctaaccgt acctcaggtg ctggctcggt gtcggacacc gcaggttcta ccgagcaagc 1080
cggcaagatc aatgtggtga aagacaccat caaggtcggc gctggcgaag tctttgacgg 1140
ccacggcgca accttcactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca 1200
gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa 1260
cgaggtcgat ggcattccag tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt 1320
15 gcattgccag aacgtcgggt aagacctgat tacggtcaaa ggcgagggag gcgcagcggg 1380
cactaatctg aacatcaaga acagcagtgc caaagggtga gacgacaagg ttgtccagct 1440
caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggg 1500
tcgcaccaac ggtggcaagc agtttgatga catgagcatt gagctgaacg gcatcgaagc 1560
taaccacggc aagttcgccc tgggtgaaaag cgacagtgac gatctgaagc tggcaacggg 1620
20 caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgaccaaca 1680
caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc 1729

```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent Application Serial No. 09/120,817 to Collmer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

```

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
30 1 5 10 15
Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
20 25 30
Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
35 35 40 45
Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
50 55 60
Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65 70 75 80
Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
40 85 90 95

```

	Ala	Asn	Lys	Thr	Gly	Asn	Val	Asp	Asp	Ala	Asn	Asn	Gln	Asp	Pro	Met	
				100					105					110			
	Gln	Ala	Leu	Met	Gln	Leu	Leu	Glu	Asp	Leu	Val	Lys	Leu	Leu	Lys	Ala	
			115					120					125				
5	Ala	Leu	His	Met	Gln	Gln	Pro	Gly	Gly	Asn	Asp	Lys	Gly	Asn	Gly	Val	
		130					135					140					
	Gly	Gly	Ala	Asn	Gly	Ala	Lys	Gly	Ala	Gly	Gly	Gln	Gly	Gly	Leu	Ala	
	145					150					155					160	
10	Glu	Ala	Leu	Gln	Glu	Ile	Glu	Gln	Ile	Leu	Ala	Gln	Leu	Gly	Gly	Gly	
				165						170					175		
	Gly	Ala	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Gly	Val	Gly	Gly	Ala	Gly	Gly	
				180					185					190			
	Ala	Asp	Gly	Gly	Ser	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Ala	Asn	Gly	Ala	
			195					200					205				
15	Asp	Gly	Gly	Asn	Gly	Val	Asn	Gly	Asn	Gln	Ala	Asn	Gly	Pro	Gln	Asn	
		210					215					220					
	Ala	Gly	Asp	Val	Asn	Gly	Ala	Asn	Gly	Ala	Asp	Asp	Gly	Ser	Glu	Asp	
	225					230					235					240	
20	Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn	
				245						250					255		
	Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Gly	Asn	Gln	
				260					265					270			
	Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly	
			275					280					285				
25	Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser	
		290					295					300					
	Gly	Gln	Asn	Asn	Leu	Gln	Ser	Gln	Ile	Met	Asp	Val	Val	Lys	Glu	Val	
	305					310					315					320	
30	Val	Gln	Ile	Leu	Gln	Gln	Met	Leu	Ala	Ala	Gln	Asn	Gly	Gly	Ser	Gln	
				325						330					335		
	Gln	Ser	Thr	Ser	Thr	Gln	Pro	Met									
				340													

Further information regarding this hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* is set forth in Arlat, M., et al., "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference in its entirety. It is encoded by a DNA molecule from *Pseudomonas solanacearum* having a nucleotide sequence corresponding SEQ. ID. No. 12 as follows:

40

```

atgtcagtcg gaaacatcca gagcccgctg aacctccggg gtctgcagaa cctgaacctc    60
aacaccaaca ccaacagcca gcaatcgggc cagtccgtgc aagacctgat caagcaggtc    120
gagaaggaca tcctcaacat catcgcagcc ctcgtgcaga aggccgcaca gtcggcgggc    180

```

	ggcaacaccg gtaacaccgg caacgcgccc gcgaaggacg gcaatgccaa cgcgggcgcc	240
	aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc	300
	ggcaacgtcg acgacgcca caaccaggat ccgatgcaag cgctgatgca gctgctggaa	360
	gacctggtga agctgctgaa ggcgccctg cacatgcagc agcccggcgg caatgacaag	420
5	ggcaacggcg tggcggtgc caacggcgcc aagggtgccg gcggccaggg cggcctggcc	480
	gaagcgctgc aggagatcga gcagatcctc gccagctcg gcggcgcgcg tctggcgcc	540
	ggcgcgcgcg gtggcggtgt cggcggtgct ggtggcgcg atggcgctc cggtcgggt	600
	ggcgcgcgcg gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac	660
	ggcccgcgaga acgcaggcga tgtcaacggg gccaacggcg cggatgacgg cagcgaagac	720
10	cagggcgggc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctggtgcag	780
	atgatgcagc aaggcgccct cggcgcgcg aaccaggcgc agggcgctc gaagggtgcc	840
	ggcaacgcct cgccggcttc cggegggaac cggggcgga accagcccgg ttcggcggt	900
	gatcaatcgt ccggccagaa caatctgcaa tccagatca tggatgtggt gaaggaggtc	960
	gtccagatcc tgcagcagat gctggcgcg cagaacggcg gcagccagca gtccacctcg	1020
15	acgcagccga tgtaa	1035

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,776,889 to Wei et al., which is hereby incorporated by reference in its entirety.

Other embodiments of the present invention include, but are not limited to, use of hypersensitive response elicitor proteins or polypeptides derived from *Erwinia carotovora* and *Erwinia stewartii*. Isolation of an *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference in its entirety. A hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference in their entirety.

Hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et

al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet, et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide which can be used in accordance with the present invention is derived from *Clavibacter michiganensis* subsp. *sepedonicus* and is described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference in its entirety.

Fragments of the above hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens can also be used according to the present invention.

Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding editions), which are hereby incorporated by reference in their entirety. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., Science 257: 85-88 (1992), which is hereby incorporated by reference in its entirety.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference in

its entirety. These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora* hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. DNA molecules encoding these fragments can also be utilized in the chimeric gene of the present invention.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the

protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

5 The hypersensitive response elicitor proteins or polypeptides used in accordance with the present invention are preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant host cells (discussed *infra*). Alternatively, the protein or polypeptide of the present invention is produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*)  
10 carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the hypersensitive response elicitor protein or polypeptide of interest is subjected to gel filtration in an appropriately sized dextran or  
15 polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC.

Other hypersensitive response elicitors can be readily identified by isolating putative protein or polypeptide candidates and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the  
20 Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using  
25 standard techniques known to those skilled in the art.

DNA molecules encoding other hypersensitive response elicitor proteins or polypeptides can also be identified by determining whether such DNA molecules hybridizes under stringent conditions to a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, 8, 10, or 12. An example of suitable  
30 stringency conditions is when hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the temperature for either hybridization or washing conditions



or increasing the sodium concentration of the hybridization or wash medium.

Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 µg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which

is hereby incorporated by reference in its entirety), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference in its entirety), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference in its entirety.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eukaryotic promoters differ from those of prokaryotic promoters. Furthermore, eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a prokaryotic system, and, further, prokaryotic promoters are not recognized and do not function in eukaryotic cells.

Similarly, translation of mRNA in prokaryotes depends upon the presence of the proper prokaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in prokaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG,

which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see  
5 Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference in its entirety.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression  
10 of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P<sub>R</sub> and P<sub>L</sub> promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*,  
15 and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which  
20 inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene  
25 transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong"  
30 transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not

limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

5                   Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast,  
10                   mammalian cells, insect, plant, and the like.

                  Because it is desirable for recombinant host cells to secrete the hypersensitive response elicitor protein or polypeptide, it is preferable that the host cell also be transformed with a type III secretion system in accordance with Ham et al., "A Cloned *Erwinia chrysanthemi* Hrp (Type III Protein Secretion) System  
15                   Functions in *Escherichia coli* to Deliver *Pseudomonas syringae* Avr Signals to Plant Cells and Secrete Avr Proteins in Culture," Microbiol. 95:10206-10211 (1998), which is hereby incorporated by reference in its entirety.

                  Isolation of the hypersensitive response elicitor protein or polypeptide from the host cell or growth medium can be carried out as described above.

20                   The methods of the present invention can be performed by treating the fruit or vegetable either prior to or after harvest of the fruit or vegetable.

                  Suitable preharvest application methods include, without limitation, high or low pressure spraying of the entire plant and fruits. Suitable postharvest application methods include, without limitation, low or high pressure spraying,  
25                   coating, or immersion. Other suitable application procedures (both preharvest and postharvest) can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with the fruit or vegetable. Once treated, the fruits or vegetables can be handled, packed, shipped, and processed using conventional procedures to deliver the produce to  
30                   processing plants or end-consumers.

                  The hypersensitive response elicitor polypeptide or protein can be applied to fruits or vegetables in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor

polypeptide or protein can be applied separately to fruits or vegetables with other materials being applied at different times.

5 A composition suitable for treating fruits or vegetables in accordance with the application embodiment of the present invention contains an isolated hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. The composition preferably contains greater than about 500 nM hypersensitive response elicitor polypeptide or protein, although greater or lesser amounts of the hypersensitive response elicitor polypeptide or protein depending on the rate of composition  
10 application and efficacy of different hypersensitive response elicitor proteins or polypeptides.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is  
15 Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and ripening agents. These materials can be used either to facilitate the process of the present invention or to provide additive benefits to inhibit postharvest disease and desiccation.

20 As indicated above, one embodiment of the present invention involves treating fruits or vegetables with an isolated hypersensitive response elicitor protein or polypeptide. The hypersensitive response elicitor protein or polypeptide can be isolated from its natural source (e.g., *Erwinia amylovora*, *Pseudomonas syringae*, etc.) or from recombinant source transformed with a DNA molecule encoding the  
25 protein or polypeptide.

Another aspect of the present invention relates to a DNA construct as well as host cells, expression systems, and transgenic plants which contain the heterologous DNA construct.

30 The DNA construct includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule and which is effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule. Expression of the DNA molecule in fruit or vegetable

tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation.

Expression of such heterologous DNA molecules requires a suitable promoter which is operable in plant tissues. In some embodiments of the present invention, it may be desirable for the heterologous DNA molecule to be expressed in many, if not all, tissues. Such promoters yield constitutive expression of coding sequences under their regulatory control. Exemplary constitutive promoters include, without limitation, the nopaline synthase promoter (Fraley et al., Proc. Natl. Acad. Sci. USA 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 35S promoter (O'Dell et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

While constitutive expression is generally suitable for expression of the DNA molecule, it should be apparent to those of skill in the art that temporally or tissue regulated expression may also be desirable, in which case any regulated promoter can be selected to achieve the desired expression. Typically, the temporally or tissue regulated promoters will be used in connection with the DNA molecule that are expressed at only certain stages of development or only in certain tissues.

In another embodiment of the present invention, expression of the heterologous DNA molecule is directed in a tissue-specific manner or environmentally-regulated manner (i.e., inducible promoters). Tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues.

For example, the E4 and E8 promoters of tomato have been used to direct fruit-specific expression of a heterologous DNA sequence in transgenic tomato plants (Cordes et al., Plant Cell 1:1025-1034 (1989); Deikman et al., EMBO J. 7:3315-3320 (1988); and Della Penna et al., Proc. Natl. Acad. Sci. USA 83:6420-6424 (1986), which are hereby incorporated by reference in their entirety). Another fruit-specific promoter is the PG promoter (Bird et al., Plant Molec. Biol. 11:651-662 (1988), which is hereby incorporated by reference in its entirety). Another tissue-specific promoter is the AP2 promoter from the ovule-specific BEL1 gene promoter

described in Reiser et al., Cell 83:735-742 (1995), which is hereby incorporated by reference in its entirety.

Promoters useful for expression in seed tissues include, without limitation, the promoters from genes encoding seed storage proteins, such as napin, cruciferin, phaseolin, and the like (see U.S. Patent No. 5,420,034 to Kridl et al., which is hereby incorporated by reference in its entirety). Other suitable promoters include those from genes encoding embryonic storage proteins.

Promoters useful for expression in leaf tissue include the Rubisco small subunit promoter.

~~Promoters useful for expression in tubers, particularly potato tubers, include the patatin promoter.~~

Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light. In some plants, it may also be desirable to use promoters which are responsive to pathogen infiltration or stress. For example, it may be desirable to limit expression of the protein or polypeptide in response to infection by a particular pathogen of the plant. One example of a pathogen-inducible promoter is the *gst1* promoter from potato, which is described in U.S. Patent Nos. 5,750,874 and 5,723,760 to Strittmayer et al., which are hereby incorporated by reference in their entirety.

Expression of the DNA molecule in isolated plant cells or tissue or whole plants also utilizes appropriate transcription termination and polyadenylation of mRNA. Any 3' regulatory region suitable for use in plant cells or tissue can be operably linked to the first and second DNA molecules. A number of 3' regulatory regions are known to be operable in plants. Exemplary 3' regulatory regions include, without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

The promoter and a 3' regulatory region can readily be ligated to the DNA molecule using well known molecular cloning techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference in its entirety.

One approach to transforming plant cells with a DNA molecule of the present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., which are hereby incorporated by reference in their entirety. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Another method of introducing the DNA molecule into plant cells is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the DNA molecule. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference in its entirety.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm, et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference in its entirety. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the DNA molecule. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.



Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with the DNA molecule. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

*Agrobacterium* is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences such as a DNA molecule a hypersensitive response elicitor protein or polypeptide can be introduced into appropriate plant cells by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. Schell, J., Science, 237:1176-83 (1987), which is hereby incorporated by reference in its entirety.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

After transformation, the transformed plant cells can be selected and regenerated.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the DNA molecule of the present invention. Suitable selection markers include, without limitation, markers coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. Cells or tissues are grown on a selection media containing an

antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a full-grown plant therefrom. Thus, another aspect of the present invention relates to a transgenic plant that includes a heterologous DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, wherein the heterologous DNA molecule is under control of a promoter that induces transcription of the DNA molecule fruit or vegetable tissues. Preferably, the DNA molecule is stably inserted into the genome of the transgenic plant of the present invention.

Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference in their entirety.

It is known that practically all plants can be regenerated from cultured cells or tissues, including both monocots and dicots.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the DNA molecule encoding the hypersensitive response elicitor protein or polypeptide is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord with common agricultural procedures known to those in the field.

With regard to the use of the hypersensitive response elicitor protein or polypeptide in imparting postharvest disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease can be reduced and symptom development can be delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of controlling postharvest disease has the potential for controlling previously untreatable diseases and avoiding the use of infectious agents or environmentally harmful materials.

With respect to desiccation, complete protection against desiccation may not be conferred, but the severity of desiccation can be reduced. Desiccation protection inevitably will depend, at least to some extent, on other conditions such as storage temperatures, light exposure, etc. However, this method of controlling desiccation has the potential for eliminating some other treatments (i.e., use of coating waxes) which may contribute to reduced costs or, at least, substantially no increase in costs.

The methods of the present invention can be used to control a number of postharvest diseases caused by a variety of pathogens. These postharvest diseases and the causative agents which can be treated according to the present invention include, without limitation, the following: *Penicillium* (e.g., *Penicillium digitatum*), *Botrytis* (e.g., *Botrytis cinerea*), *Phytophthora* (e.g., *Phytophthora citrophthora*), and *Erwinia* (e.g. *Erwinia carotovora*).

A further aspect of the present invention relates to a method of enhancing the longevity of fruit or vegetable ripeness.

According to one embodiment, this aspect of the present invention is carried out by treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhance the longevity of fruit or vegetable ripeness. Preferably, as noted above, the hypersensitive response elicitor protein or polypeptide is in isolated form. Treating of the fruit or vegetable can be performed either prior to harvest after harvest of the fruit or vegetable, using the techniques described above.

According to another embodiment, this aspect of the present invention is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and then growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to enhance the longevity of fruit or vegetable ripeness in a fruit or vegetable harvested from the transgenic plant. This aspect of the present invention may further include applying the hypersensitive response elicitor polypeptide or protein to the fruit or vegetable to enhance the longevity of fruit or vegetable ripeness. Treating of the fruit or vegetable can be performed either prior to harvest or after harvest of the fruit or vegetable, using the techniques described above.

The methods of the present invention can be utilized to treat a wide variety of fruits and vegetables to control postharvest disease or desiccation as well as enhance the longevity of fruit or vegetable ripeness. Fruits and vegetables which can be treated include any edible plant product, particularly those from traditional crop plants, such as seed, root, tuber, stem, leaf, flower, and fruit. Exemplary transgenic fruit plants and fruits that can be treated include, without limitation, apple, pear, peach, nectarine, apricot, plum, cherry, olive, melon, citrus, grape, strawberry, raspberry, blueberry, currant, pineapple, papaya, guava, banana, and kiwi. Exemplary transgenic vegetable plants and vegetables that can be treated include, without limitation, asparagus, potato, sweet potato, bean, pea, chicory, lettuce, parsley, basil, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, spinach, onion, garlic, eggplant, pepper, celery, leek, radish, carrot, squash, pumpkin, zucchini, cucumber, soybean, tobacco, tomato, sorghum, rhubarb, and sugarcane. Exemplary transgenic grain plants and grain products which can be treated include, without limitation, alfalfa, rice, wheat, barley, corn, and rye.

### EXAMPLES

The following examples are provided to illustrate embodiments of the present invention but are by no means intended to limit its scope.

As used in the following Examples, Messenger<sup>®</sup> refers to a product available from Eden Bioscience Corporation (Bothell, Washington), which contains 3% by weight of harpin<sub>Ea</sub> as the active ingredient and 97% by weight inert ingredients. Harpin<sub>Ea</sub> is one type of hypersensitive response elicitor protein from *Erwinia amylovora*, identified herein by SEQ. ID. No. 3.

**Example 1 – Effect of Treating Orange Fruits with Messenger® on Postharvest Orange Storage**

On day 0, Fall-GLO orange fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on oranges were then dried by air, and the treated oranges were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated oranges was then put into a 18°C growth chamber for storage. On day 7, orange fruits were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of orange fruit. The above procedure was performed on 40 orange fruits per treatment.

Measurements of disease were conducted on days 20, 24, and 26 following treatment with Messenger® or buffer solution. Grades 0-5 indicate different disease scales -- Grade 0: No symptoms; Grade 1: 1/5 an individual fruit has disease symptoms; Grade 2: 2/5 an individual fruit has disease symptoms; Grade 3: 3/5 an individual fruit has disease symptoms; Grade 4: 4/5 an individual fruit has disease symptoms; Grade 5: whole fruit has disease symptoms. The results of these treatments are set forth in Table 1 below.

Table 1: Reduction of Disease Index in Oranges

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger®	20	33	3	1	0	2	1	0.09	58.14%	yes	yes
Buffer	20	23	8	0	2	6	1	0.22	n/a	-	-
Messenger®	24	25	2	6	4	1	2	0.20	45.21%	yes	yes
Buffer	24	16	7	3	3	4	7	0.37	n/a	-	-
Messenger®	26	19	4	6	4	5	2	0.29	36.96%	yes	yes
Buffer	26	16	3	3	0	7	11	0.46	n/a	-	-

The data listed in Table 1 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger® treatment can reduce orange disease about 58.14% at 21 days, about 45.21% at 25 days, and 36.97% at 27 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95%

and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 2 – Effect of Treating Tomato (Hot House) Fruits with Messenger<sup>®</sup> on Postharvest Tomato Storage**

On day 0, Hot House tomato fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger<sup>®</sup> or buffer solutions on tomatoes were then dried by air, and the treated tomatoes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated tomatoes was then put into 18°C growth chamber for storage. On day 7, tomatoes were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of tomato fruit. The above procedure was performed on 15 tomatoes fruits per treatment.

Measurements of disease were conducted on days 21 and 27 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 2 below.

Table 2: Reduction of Disease Index in Tomatoes

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger <sup>®</sup>	21	7	2	2	3	1	0	0.25	58.70%	yes	yes
Buffer	21	3	1	2	1	2	6	0.61	n/a	-	-
Messenger <sup>®</sup>	27	2	2	4	3	2	2	0.49	30.19%	yes	yes
Buffer	27	1	1	2	2	3	6	0.71	n/a	-	-

The data listed in Table 2 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger<sup>®</sup> treatment can reduce tomato disease about 58.70% at 21 days and about 30.19% at 27 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 3 – Effect of Treating Grape Fruits with Messenger® on Postharvest Grape Storage**

On day 0, Red G. Grape fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on grapes were then dried by air, and the treated grapes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated grapes was then put into a 18°C growth chamber for storage. On day 7, grapes were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of grape fruit. The above procedure was performed on about 3700g of grape fruits per treatment.

Measurements of disease were conducted on days 14 and 21 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 3 below.

Table 3: Reduction of Disease Index in Grapes

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger®	14	225	99	42	39	21	13	0.20	45.65%	yes	yes
Buffer	14	98	130	91	52	38	48	0.38	n/a	-	-
Messenger®	21	66	83	126	98	39	27	0.42	39.35%	yes	yes
Buffer	21	18	36	64	72	119	137	0.69	n/a	-	-

The data listed in Table 3 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger® treatment can reduce grape disease by about 45.65% at 14 days and about 39.35% at 21 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

**Example 4 – Effect of Treating Grapefruit Fruits with Messenger® on Postharvest Grapefruit Storage**

On day 0, FL 33935 grapefruit fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on grapefruits were then dried by air, and the treated grapefruits were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated grapefruit fruits was then put into a 18°C growth chamber for storage. On day 7, grapefruit fruits were inoculated with *Phytophthora citrophthora* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of grapefruit fruit. The above procedure was performed on 6 grapefruit fruits per treatment.

Measurements of disease were conducted on days 87, 97, 103, and 111 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 4 below.

Table 4: Reduction of Disease Index in Grapefruits

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger®	87	5	1	0	0	0	0	0.03	75.00%	yes	yes
Buffer	87	4	1	0	1	0	0	0.13	n/a	-	-
Messenger®	97	5	0	0	1	0	0	0.10	50.00%	yes	yes
Buffer	97	4	0	1	0	1	0	0.20	n/a	-	-
Messenger®	103	4	1	0	0	1	0	0.17	28.57%	yes	yes
Buffer	103	3	2	0	0	0	1	0.23	n/a	-	-
Messenger®	111	4	1	0	0	0	1	0.20	33.33%	yes	yes
Buffer	111	3	1	0	1	0	1	0.30	n/a	-	-

The data listed in Table 4 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Phytophthora citrophthora* and providing longer storage life. Messenger® treatment can reduce grapefruit disease by about 75.00% at 87 days, about 50.00% at 97 days, about 28.57% at 103 days, and about 33.33% at 111 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant



differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 5 - Effect of Treating Apple (Fuji) Fruits with Messenger<sup>®</sup> on Postharvest Apple Storage**

On day 0, Fuji apple fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger<sup>®</sup> or buffer solutions on apples were then dried by air, and the treated apples were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated apples was then put into a 18°C growth chamber for storage. On day 7, apples were inoculated with *Penicillium digitatum* and *Phytophthora citrophora* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of apples. The above procedure was performed on 20 apples per treatment.

Measurements of disease were conducted on days 50, 61, 70, 78, and 85 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 5 below.

Table 5: Reduction of Disease Index in Apples

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger <sup>®</sup>	50	20	0	0	0	0	0	0.00	100.00%	yes	yes
Buffer	50	18	1	1	0	0	0	0.03	n/a	-	-
Messenger <sup>®</sup>	61	19	1	0	0	0	0	0.01	88.89%	yes	yes
Buffer	61	16	2	1	0	0	1	0.09	n/a	-	-
Messenger <sup>®</sup>	70	18	0	2	0	0	0	0.04	71.43%	yes	yes
Buffer	70	14	2	2	1	0	1	0.14	n/a	-	-
Messenger <sup>®</sup>	78	15	2	3	0	0	0	0.08	57.89%	yes	yes
Buffer	78	13	2	2	1	0	2	0.19	n/a	-	-
Messenger <sup>®</sup>	85	13	3	1	1	2	0	0.16	40.74%	yes	yes
Buffer	85	10	5	0	0	3	2	0.27	n/a	-	-

The data listed in Table 5 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Phytophthora citrophora* and providing longer storage life.

Messenger<sup>®</sup> treatment can reduce apple disease by about 100.00% at 51 days, 88.89% at 61 days, 71.43% at 70 days, 57.89% at 78 days, and 40.74% at 85 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 6 - Effect of Treating Apple (Granny Smith) Fruits with Messenger<sup>®</sup> on Postharvest Apple Storage**

On day 0, Granny Smith apple fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger<sup>®</sup> or buffer solutions on apples were then dried by air, and the treated apples were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated apples was then put into a 18°C growth chamber for storage. On day 7, apples were inoculated with *Penicillium digitatum* and *Phytophthora citrophora* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of apples. The above procedure was performed on 20 apples per treatment.

Measurements of disease were conducted on days 50, 61, 70, 78, and 85 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 6 below.

Table 6: Reduction of Disease Index in Apples

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger <sup>®</sup>	50	20	0	0	0	0	0	00.00	100.00%	yes	yes
Buffer	50	19	1	0	0	0	0	0.01	n/a	-	-
Messenger <sup>®</sup>	61	13	5	2	0	0	0	0.09	50.00%	yes	yes
Buffer	61	7	9	3	1	0	0	0.18	n/a	-	-
Messenger <sup>®</sup>	70	7	10	3	0	0	0	0.16	36.00%	yes	yes
Buffer	70	2	12	5	1	0	0	0.25	n/a	-	-
Messenger <sup>®</sup>	78	6	10	3	1	0	0	0.19	32.14%	yes	yes
Buffer	78	2	11	5	1	1	0	0.28	n/a	-	-
Messenger <sup>®</sup>	85	7	9	2	1	1	0	0.20	23.08	yes	yes
Buffer	85	4	10	4	1	0	1		n/a	-	-

The data listed in Table 6 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Phytophthora citrophora* and providing longer storage life. Messenger<sup>®</sup> treatment can reduce apple disease by about 100.00% at 51 days, 50.00% at 61 days, 36.00% at 70 days, 32.14% at 78 days, and 23.08% at 85 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

#### 10 Example 7 - Effect of Treating Tomato Fruits with Messenger<sup>®</sup> on Postharvest Tomato Storage

On day 0, tomato fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. After the Messenger<sup>®</sup> or buffer solutions on tomatoes were dried by air, the treated tomatoes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated tomatoes was then put into a 18°C growth chamber for storage. On day 7, tomatoes were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of tomatoes. The above procedure was performed on 44 tomatoes per treatment.

Measurements of disease were conducted on days 18, 27, 35, and 42 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 7 below.

Table 7: Reduction of Disease Index in Tomatoes

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger <sup>®</sup>	18	21	18	5	0	0	0	0.13	37.78%	yes	yes
Buffer	18	11	21	12	0	0	0	0.20	n/a	-	-
Messenger <sup>®</sup>	27	16	18	9	1	0	0	0.18	25.00%	yes	yes
Buffer	27	8	24	8	4	0	0	0.24	n/a	-	-
Messenger <sup>®</sup>	35	7	14	13	10	0	0	0.32	16.67%	yes	yes
Buffer	35	1	16	15	10	2	0	0.38	n/a	-	-
Messenger <sup>®</sup>	42	1	10	9	12	9	3	0.52	12.88%	yes	yes
Buffer	42	0	3	15	10	11	5	0.60	n/a	-	-

The data listed in Table 7 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger<sup>®</sup> treatment can reduce tomato disease by about 37.78% at 18 days, 25.00% at 27 days, 16.67% at 35 days, and 12.88% at 42 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger<sup>®</sup> treatment and buffer treatment.

**Example 8 - Effect of Preharvest and Postharvest Messenger<sup>®</sup> Treatments on Tomato (Sanibel) Fruit Postharvest Storage**

Plots of red and green Sanibel variety tomatoes were grown under either standard conditions or full Messenger<sup>®</sup> treatment over the course of the growing season. The standard conditions, also known as grower's standard, included fungicide treatment sprayed every seven days after transplanting using primarily fungicides containing copper-based active ingredients. The Messenger<sup>®</sup> treatment included six sprays at rate of 2.2 oz of the product per acre.

Red and green fruits were harvested from both the Messenger<sup>®</sup> treated and grower standard plots. It was noted that green tomatoes from the grower standard treatment plots were smaller (i.e. less mature) than green tomatoes from the messenger treated plants.

Harvested fruits were treated as follows:

- (1) Fruits from Messenger<sup>®</sup> treated plots were further treated with Messenger<sup>®</sup> after harvest;
- (2) Fruits from standard plots were treated with Messenger<sup>®</sup> after harvest;
- (3) Fruits from Messenger<sup>®</sup> treated plots received no additional treatment following harvest; and
- (4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (1) and (2) was carried out by spraying with Messenger<sup>®</sup> at a rate of 20 ppm harpin<sub>Ea</sub> concentration using a

backpack-sprayer at about 30 p.s.i. The fruit were rolled during application to assure full coverage of the spray. The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in storage in a single layer. Storage temperatures ranged from about 18 to 32°C and light intervals were approximately 12 hours of light and darkness. Tomatoes were checked daily for rot and desiccation for a total of 31 days after harvest. The results are shown in Table 8 below.

Table 8: Affect of Preharvest and Postharvest Treatment on Rot and Desiccation

Group	Ripe- ness	No. Fruit	Days After Harvest								No. Desiccated	% Marketable
			14	19	21	22	23	25	31			
(1) Pre/Postharvest Messenger®	Red	5	0	0	0	1	1	1	2	0	60%	
	Green	4	0	0	0	0	0	0	0	0	100%	
(2) Postharvest Messenger® Only	Red	5	0	0	0	0	0	0	0	4	20%	
	Green	4	0	0	0	0	0	0	0	1	75%	
(3) Preharvest Messenger® Only	Red	5	0	0	0	0	0	0	2	0	60%	
	Green	5	0	0	0	0	0	0	0	0	100%	
(4) No Messenger®	Red	5	1	3	1	5	5	5	5	0	0%	
	Green	5	0	0	0	0	0	0	0	1	80%	

The red tomatoes from group (4) all rotted by day 21. In contrast, all red tomatoes which received some form of Messenger® treatment showed reduced rate of decay and rot. Near the end of the trial a number of tomatoes were observed to have desiccated, exhibiting shriveled skins but no rot. These were included as non-marketable. These results are suggestive that both preharvest and postharvest Messenger® treatments can reduce the level of rotting and desiccation, thereby extending fresh storage time.

#### **Example 9 - Effect of Messenger on Post Harvested Maturity and Fruit Decay on Tomato During Ambient Storage**

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger® treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Three different treatment regimen were examined as follows:

- 5
- (1) Fruits from Messenger<sup>®</sup> treated plots received no additional treatment following harvest;
  - (2) Fruits from standard plots were treated with Messenger<sup>®</sup> after harvest;
  - (3) Fruits from standard plots received no additional treatment following harvest.

10 Postharvest treatment of fruits from group (2) was carried out by dipping the fruit in a Messenger<sup>®</sup> solution (20 ppm harpin<sub>EA</sub>). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(3) were marked and mixed together in tomato crates for storage. Storage temperatures ranged from about 23 to 26°C (75-80°F). The tomatoes were then rated for color development and decay over time using the rating scale below.

	<u>Grade</u>	<u>Description</u>
15	1	Mature Green: When fruit cut in half, no seeds cut; fruit entirely green with no color break;
	2	Pink: Initial sign of color break noticed on some areas of fruit; these areas are usually pink;
	3	Pink/Red: Intermediate ripening: Fruit is not total red; some pink still remains;
20	4	Red: Fruit totally red in color;
	5	Decay: Some areas of the fruit beginning to break down from decay.

The results of this test are summarized in Table 9 below.

Table 9: Affect of Preharvest and Postharvest Treatment on Maturity and Decay

Group	Days After Treatment	Grade					Index	Efficacy	T-test	
		1	2	3	4	5			p<0.05	p<0.01
1	10	11	6	8	75	0	0.69	7.28%	yes	yes
2	10	5	7	11	77	0	0.72	3.81%	yes	yes
3	10	5	3	6	86	1	0.75	N/A	N/A	N/A
1	14	4	5	5	86	0	0.75	2.61%	yes	yes
2	14	2	6	5	87	0	0.75	1.57%	yes	yes
3	14	2	4	4	89	1	0.77	N/A	N/A	N/A
1	17	0	0	3	92	5	0.80	3.37%	yes	yes
2	17	0	1	4	82	13	0.81	2.16%	yes	yes
3	17	0	0	1	82	17	0.83	N/A	N/A	N/A
1	20	0	0	0	89	11	0.82	2.61%	yes	yes
2	20	0	0	0	80	20	0.84	0.47%	yes	yes
3	20	0	0	1	76	23	0.84	N/A	N/A	N/A

The data generated in this trial indicate that treatment of tomatoes with Messenger<sup>®</sup>, either through field sprays or as a post harvest dip, resulted in earlier fruit red ripening compared to grower's standard. In addition, although early ripening was observed, the Messenger<sup>®</sup> treatments maintained the red ripe condition longer than the grower's standard with delay of breakdown and decay.

#### **Example 10 - Effect on Messenger on Post Harvested Maturity and Fruit Decay of Tomato Under Cold Storage Conditions**

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger<sup>®</sup> treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Four different treatment regimen were examined as follows:

- (1) Fruits from Messenger<sup>®</sup> treated plots received no additional treatment following harvest;
- (2) Fruits from Messenger<sup>®</sup> treated plots were further treated with Messenger<sup>®</sup> after harvest;

- (3) Fruits from standard plots were treated with Messenger® after harvest; and
- (4) Fruits from standard plots received no additional treatment following harvest.

5 Postharvest treatment of fruits from groups (2) and (3) were carried out by dipping the fruit in a Messenger® solution (20 ppm harpin<sub>Ea</sub>). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in tomato crates for storage in a Custom Packing House cooler at 11°C (52°F). The tomatoes were then rated for color development and

10 decay over time using the rating scale described in Example 8. The results of this study appear in Table 10 below.

Table 10: Affect of Preharvest and Postharvest Treatment on Maturity and Decay

Group	Days After Treatment	Grade					Index	Efficacy	T-test	
		1	2	3	4	5			p<0.05	p<0.01
1	7	66	34	0	0	0	0.27	0.00%	yes	yes
2	7	67	33	0	0	0	0.27	0.75%	yes	yes
3	7	76	24	0	0	0	0.27	7.46%	yes	yes
4	7	68	30	2	0	0	0.27	N/A	yes	yes
1	10	59	31	8	0	0	0.30	7.53%	yes	yes
2	10	60	28	12	0	0	0.30	5.00%	yes	yes
3	10	65	35	0	0	0	0.27	15.63%	yes	yes
4	10	49	42	9	0	0	0.32	N/A	N/A	N/A
1	17	19	35	28	18	0	0.49	7.20%	yes	yes
2	17	20	38	28	14	0	0.47	10.61%	yes	yes
3	17	19	28	39	14	0	0.50	6.06%	yes	yes
4	17	17	27	31	25	0	0.53	N/A	N/A	N/A
1	21	11	28	29	32	0	0.56	6.62%	N/A	N/A
2	21	15	26	37	22	0	0.53	11.92%	yes	yes
3	21	10	33	35	22	0	0.54	10.93%	yes	yes
4	21	10	18	32	40	0	0.60	N/A	N/A	N/A
1	26	3	15	23	59	0	0.68	-2.26%	yes	yes
2	26	9	19	25	41	6	0.63	4.39%	yes	yes
3	26	3	23	31	43	0	0.63	5.00%	yes	yes
4	26	2	19	23	50	1	0.66	N/A	N/A	N/A
1	32	3	15	23	59	0	0.68	-2.26%	yes	yes
2	32	9	19	25	41	6	0.63	4.39%	yes	yes
3	32	3	23	31	43	0	0.63	5.00%	yes	yes
4	32	2	19	23	50	1	0.66	N/A	N/A	N/A



Table 10 cont.

Group	Days After Treatment	Grade					Index	Efficacy	T-test	
		1	2	3	4	5			p<0.05	p<0.01
1	38	0	4	10	84	2	0.77	0.26%	yes	yes
2	38	1	10	15	65	9	0.74	3.64%	yes	yes
3	38	1	5	14	78	2	0.75	2.60%	yes	yes
4	38	0	3	13	80	4	0.77	N/A	N/A	N/A
1	45	0	3	11	74	12	0.79	2.95%	yes	yes
2	45	1	4	12	69	14	0.78	3.93%	yes	yes
3	45	0	1	11	81	7	0.79	3.19%	yes	yes
4	45	0	0	10	73	17	0.81	N/A	N/A	N/A
1	50	0	3	10	63	23	0.82	3.55%	yes	yes
2	50	0	4	11	58	27	0.82	3.55%	yes	yes
3	50	0	0	8	78	14	0.81	4.02%	yes	yes
4	50	0	0	3	71	26	0.85	N/A	N/A	N/A
1	55	0	0	0	73	27	0.85	1.84%	yes	yes
2	55	0	0	0	68	32	0.86	0.69%	yes	yes
3	55	0	0	2	80	18	0.83	4.37%	yes	yes
4	55	0	0	0	65	35	0.87	N/A	N/A	N/A
1	60	0	0	0	65	35	0.87	2.47%	yes	yes
2	60	0	0	0	63	37	0.87	2.02%	yes	yes
3	60	0	0	0	74	26	0.85	4.48%	yes	yes
4	60	0	0	0	54	46	0.89	N/A	N/A	N/A
1	65	0	0	0	53	47	0.89	1.76%	yes	yes
2	65	0	0	0	58	42	0.88	2.86%	yes	yes
3	65	0	0	0	65	35	0.87	4.40%	yes	yes
4	65	0	0	0	45	55	0.91	N/A	N/A	N/A

5 In previous trials when tomatoes were treated with Messenger® in the field and/or with a post harvest dip, the fruit appeared to develop to red ripe more quickly than the grower's standard, when held at ambient temperatures (75-80°F). Although this early ripening was observed, these red fruit did not begin to decay earlier than the grower's standard. In this study, the fruit were held at a constant 52°F in a commercial cold storage room at a tomato packinghouse facility. It appears that

10 this lower temperature slows the ripening process, as would be expected, and Messenger® treatments did not increase the rate of the red ripening for the first 30 days, as observed in previous tests. The Messenger® treatments did, however,

seem to maintain the red ripe condition longer than the grower's standard without breakdown and decay.

**Example 11 - Effect of Messenger on Post Harvested Maturity and Fruit Decay on Tomato**

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger<sup>®</sup> treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Four different treatment regimen were examined as follows:

- (1) Fruits from Messenger<sup>®</sup> treated plots received no additional treatment following harvest;
- (2) Fruits from Messenger<sup>®</sup> treated plots were further treated with Messenger<sup>®</sup> after harvest;
- (3) Fruits from standard plots were treated with Messenger<sup>®</sup> after harvest; and
- (4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (2) and (3) were carried out by dipping the fruit in a Messenger<sup>®</sup> solution (20 ppm harpin<sub>EA</sub>). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in tomato crates for storage. Storage temperatures ranged from about 23 to 26°C (75-80°F). The tomatoes were then rated for color development and decay over time using the commercial rating scale from the Florida Tomato Committee color guide as follows:

**Grade Description**

- |   |  |
|---|--|
| 1 | Green: When fruit cut in half, no seeds cut; fruit entirely green with no color break;                   |
| 2 | Breakers: Initial sign of color break on 10% or less of the area of fruit; these areas are usually pink; |
| 3 | Turning: Pink or red on 10 to 30% of the fruit surface;  |

- 4 Pink: Pink or red on 30 to 60% of the fruit surface;  
 5 Light Red: Pink on over 60% of fruit surface and red color no more  
 than 90% of fruit surface;  
 6 Red: Fruit totally red in color; and  
 7 Decay: Some areas of the fruit beginning to break down from decay.

The results of this treatment are set forth in Table 11 below.

Table 11: Affect of Preharvest and Postharvest Treatment on Maturity and Decay Data

Group	Days After Treatment	Grade							Index	Efficacy	T-test	
		1	2	3	4	5	6	7			p<0.05	p<0.01
1	3	80	18	2	0	0	0	0	0.17	0.00%	no	no
2	3	73	17	9	1	0	0	0	0.20	-13.11%	yes	yes
3	3	78	19	3	0	0	0	0	0.18	-2.46%	yes	yes
4	3	80	18	2	0	0	0	0	0.17	N/A	no	no
1	7	36	23	22	12	5	2	0	0.33	3.72%	yes	no
2	7	37	23	17	19	4	0	0	0.33	4.96%	yes	no
3	7	40	17	15	18	9	1	0	0.35	0.00%	yes	no
4	7	35	22	19	15	8	1	0	0.35	N/A	no	no
1	14	2	5	8	8	13	65	0	0.74	8.02%	yes	yes
2	14	2	3	5	9	8	72	1	0.77	4.44%	yes	yes
3	14	4	4	7	8	17	60	0	0.73	9.41%	yes	yes
4	14	0	0	6	5	13	72	4	0.80	N/A	no	no
1	17	0	0	2	3	6	89	0	0.83	2.51%	yes	yes
2	17	1	1	1	0	7	88	2	0.83	2.35%	yes	yes
3	17	1	2	0	0	9	88	0	0.83	3.18%	yes	yes
4	17	0	0	0	0	7	89	4	0.85	N/A	no	no
1	21	0	0	0	0	0	97	3	0.86	1.31%	yes	yes
2	21	0	0	0	0	0	97	3	10.86	1.31%	yes	yes
3	21	0	0	0	0	3	95	2	0.86	1.96%	yes	yes
4	21	0	0	0	0	1	87	12	0.87	N/A	no	no
1	28	0	0	0	0	0	85	15	0.88	2.84%	yes	yes
2	28	0	0	0	0	0	91	9	0.87	3.79%	yes	yes
3	28	0	0	0	0	0	81	19	0.88	2.21%	yes	yes
4	28	0	0	0	0	0	67	33	0.90	N/A	no	no
1	32	0	0	0	0	0	22	78	0.97	2.16%	yes	yes
2	32	0	0	0	0	0	16	84	0.98	1.30%	yes	yes
3	32	0	0	0	0	0	55	45	0.92	6.93%	yes	yes
4	32	0	0	0	0	0	7	93	0.99	N/A	no	no

Table 11 Cont.

Group	Days After Treatment	Grade							Index	Efficacy	T-test	
		1	2	3	4	5	6	7			p<0.05	p<0.01
1	37	0	0	0	0	0	14	86	0.98	1.15%	yes	yes
2	37	0	0	0	0	0	7	93	0.00	0.14%	yes	yes
3	37	0	0	0	0	0	9	91	0.99	0.43%	yes	yes
4	37	0	0	0	0	0	6	94	0.99	N/A	no	no
1	42	0	0	0	0	0	12	88	0.98	1.01%	yes	yes
2	42	0	0	0	0	0	7	93	0.99	0.29%	yes	yes
3	42	0	0	0	0	0	8	92	0.99	0.43%	yes	yes
4	42	0	0	0	0	0	5	95	0.99	N/A	no	no
1	45	0	0	0	0	0	8	92	0.99	0.57%	no	no
2	45	0	0	0	0	0	4	96	0.99	0.00%	no	no
3	45	0	0	0	0	0	4	96	0.99	0.00%	no	no
4	45	0	0	0	0	0	4	96	0.99	N/A	no	no
1	50	0	0	0	0	0	7	93	0.99	0.43%	no	no
2	50	0	0	0	0	0	4	96	0.99	0.00%	no	no
3	50	0	0	0	0	0	4	96	0.99	0.00%	no	no
4	50	0	0	0	0	0	4	96	0.99	N/A	no	no

In previous trials tomatoes treated with Messenger<sup>®</sup> in the field and/or with a post harvest dip appeared to develop to red ripe more quickly, but decayed slower than the grower's standard. The data generated from this trial support these observations. By twenty-one days post harvest, 97% of the Messenger<sup>®</sup> treated tomatoes were full red ripe, compared to 87% of the grower's standard. Although it may be assumed that fruit which reach maturity more quickly will also start to break down more quickly, the results of the present Examples surprisingly demonstrate that these earlier-maturing tomatoes were actually 15% slower to decay than the grower's standard tomatoes. This phenomenon should be of great interest of several segments of the tomato market. The growers may be able to reduce ethylene gashouse timings, and the retail market should be able to significantly reduce inventory shrinkage.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

## WHAT IS CLAIMED:

1. A method of inhibiting postharvest disease or desiccation in a fruit or vegetable, said method comprising:

treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

2. The method according to claim 1, wherein hypersensitive response-elicitor-protein or polypeptide is in isolated form.

3. The method according to claim 2, wherein said treating is carried out prior to harvest of the fruit or vegetable.

4. The method according to claim 3, wherein said treating is carried out by spraying the fruit or vegetable with the hypersensitive response elicitor protein or polypeptide.

5. The method according to claim 4, wherein the hypersensitive response elicitor protein or polypeptide is in liquid or powder form.

6. The method according to claim 1, wherein said treating is carried out after harvest of the fruit or vegetable.

7. The method according to claim 6, wherein said treating is carried out by spraying the fruit or vegetable with the hypersensitive response elicitor protein or polypeptide.

8. The method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is in liquid or powder form.

9. The method according to claim 6, wherein said treating is carried out by immersing the fruit or vegetable in the hypersensitive response elicitor protein or polypeptide.

5 10. The method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

10 11. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

12. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

15 13. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

20 14. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

15. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

25 16. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

17. The method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of *Phytophthora*.

30 18. The method according to claim 1, wherein said treating inhibits desiccation in a fruit or vegetable.

19. The method according to claim 1, wherein said treating inhibits a postharvest disease in a fruit or vegetable.

20. The method according to claim 19, wherein the postharvest disease is caused by *Penicillium*, *Botrytis*, *Phytophthora*, or *Erwinia*.

21. A method of inhibiting postharvest disease or desiccation in a fruit or vegetable, said method comprising:

providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

22. The method according to claim 21, wherein a transgenic plant is provided.

23. The method according to claim 21, wherein a transgenic plant seed is provided.

24. The method according to claim 21, wherein the transgenic plant is a dicot or a monocot.

25. The method according to claim 21, further comprising:  
applying the hypersensitive response elicitor polypeptide or protein to the fruit or vegetable to inhibit postharvest disease or desiccation.

26. The method according to claim 25, wherein said applying is carried out prior to harvest of the fruit or vegetable.

27. The method according to claim 25, wherein said applying is carried out after harvest of the fruit or vegetable.

28. The method according to claim 21, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

5

29. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

10

30. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

31. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

15

32. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

33. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

20

34. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

25

35. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of *Phytophthora*.

36. The method according to claim 21, wherein the postharvest disease is caused by *Penicillium*, *Botrytis*, *Phytophthora*, or *Erwinia*.



37. A DNA construct comprising:  
a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide;  
a plant-expressible promoter operably coupled 5' to the DNA molecule, the promoter being effective to transcribe the DNA molecule in fruit or vegetable tissue; and  
a 3' regulatory region operably coupled to the DNA molecule, wherein expression of the DNA molecule in fruit or vegetable tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation.
38. An expression system comprising a vector into which is inserted a heterologous DNA construct according to claim 37.
39. A host cell comprising a heterologous DNA construct according to claim 37.
40. The host cell according to claim 39, wherein the host cell is a plant cell or a bacteria cell.
41. The host cell according to claim 40, wherein the bacteria cell is an *Agrobacterium* cell.
42. A transgenic plant comprising a heterologous DNA construct according to claim 37.
43. A method of enhancing the longevity of fruit or vegetable ripeness comprising:  
treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhance the longevity of fruit or vegetable ripeness.

**THIS PAGE BLANK (USPTO)**

44. The method according to claim 43, wherein hypersensitive response elicitor protein or polypeptide is in isolated form.

5 45. The method according to claim 43, wherein said treating is carried out prior to harvest of the fruit or vegetable.

46. The method according to claim 43, wherein said treating is carried out after harvest of the fruit or vegetable.

10 47. A method of enhancing the longevity of fruit or vegetable ripeness comprising:  
providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and  
growing the transgenic plant or transgenic plant produced from  
15 the transgenic plant seed under conditions effective to enhance the longevity of fruit or vegetable ripeness in a fruit or vegetable harvested from the transgenic plant.

48. The method according to claim 47, further comprising:  
applying the hypersensitive response elicitor polypeptide or  
20 protein to the fruit or vegetable to enhance the longevity of fruit or vegetable ripeness.

49. The method according to claim 48, wherein said applying is carried out prior to harvest of the fruit or vegetable.

25 50. The method according to claim 48, wherein said applying is carried out after harvest of the fruit or vegetable.

## SEQUENCE LISTING

&lt;110&gt; Eden Bioscience Corporation

<120> TREATMENT OF FRUITS OR VEGETABLES WITH HYPERSENSITIVE  
RESPONSE ELICITOR TO CONTROL POSTHARVEST DISEASE OR  
DESICCATION

&lt;130&gt; 21829/72

&lt;140&gt;

&lt;141&gt;

&lt;150&gt; 60/198,359

&lt;151&gt; 2000-04-19

&lt;160&gt; 12

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 338

&lt;212&gt; PRT

&lt;213&gt; Erwinia chrysanthemi

&lt;400&gt; 1

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser  
1 5 10 15Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser  
20 25 30Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr  
35 40 45Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu  
50 55 60Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser  
65 70 75 80Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys  
85 90 95Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp  
100 105 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln

**THIS PAGE BLANK (USPTO)**

115	120	125
Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met		
130	135	140
Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly		
145	150	155
Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly		
	165	170
Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu		
	180	185
Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala		
	195	200
Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val		
	210	215
Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp		
	225	230
Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp		
	245	250
Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys		
	260	265
Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln		
	275	280
Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr		
	290	295
Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala		
	305	310
Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala		
	325	330

Asn Ala

&lt;210&gt; 2

&lt;211&gt; 2141

&lt;212&gt; DNA

**THIS PAGE BLANK (USPTO)**

<213> *Erwinia chrysanthemi*

&lt;400&gt; 2

```

cgattttacc cgggtgaacg tgctatgacc gacagcatca cggatttcga caccgttacg 60
gcgtttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgccgca atccggcgctc 120
gatctggtat ttcagtttg ggacaccggg cgtgaactca tgatgcagat tcagccgggg 180
cagcaatata ccggcatggt gcgcacgctg ctcgctcgct gttatcagca ggccggcagag 240
tgcgatggct gccatctgtg cctgaacggc agcgatgtat tgatcctctg gtggccgctg 300
ccgtcggatc ccggcagtta tccgcaggtg atcgaacggt tgtttgaact ggccgggaatg 360
acgttgccgt cgctatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc 420
cgatcattaa gataaaggcg gcttttttta ttgcaaaacg gtaacggtga ggaaccgttt 480
caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgctg 540
ggcatccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca 600
aattacgata aaagcgcaca tcggcggtga tttggcgctc tccggtctgg ggctgggtgc 660
tcagggactg aaaggactga attccgcggc ttcctcgctg ggttccagcg tggataaact 720
gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct 780
ggcgcgaggg ctggcgcgcca gctcgaaggg gctggggatg agcaatcaac tgggccagtc 840
tttcggcaat ggcgcgcgagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcgcgga 900
tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac 960
caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020
ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcaactgctg ccattctcgg 1080
caacggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggtt 1140
gcagggcctg agcgcgcgcg gtgcattcaa ccagttgggt aatgccatcg gcatggcgct 1200
ggggcagaat gctgcgctga gtgcgttag taacgtcagc acccagtag acggtaacaa 1260
ccgccacttt gtagataaag aagatcgcg catggcgaaa gagatcggcc agtttatgga 1320
tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380
gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg 1440
cgccagcatg gacaaattcc gtcaggcgat gggtatgac aaaagcgcgg tggcggtga 1500
taccggcaat accaactga acctgcgtgg cgcgggcggt gcatcgctgg gtatcgatgc 1560
ggctgtcgct ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620
atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680
ttattatgcg gtttatgagg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740
acgcacattt tcccgttcat tcgcgtcggt acgcgccaca atcgcgatgg catcttcctc 1800
gtcgctcaga ttgcgcgggt gatggggaac gccgggtgga atatagagaa actcgccggc 1860
cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gcccttttag 1920
cagatagatt gcggtttcgt aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980
gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac 2040
aaaatagggc agtttttgcg tggtatccgt ggggtgttcc ggcctgacaa tcttgagttg 2100
gttcgtcatc atctttctcc atctggcgga cctgatcggt t 2141

```

&lt;210&gt; 3

&lt;211&gt; 403

&lt;212&gt; PRT

<213> *Erwinia amylovora*

&lt;400&gt; 3

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser

**THIS PAGE BLANK (USPTO)**



1	5	10	15
Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln	20	25	30
Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn	35	40	45
Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met	50	55	60
Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu	65	70	75
Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu	85	90	95
Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr	100	105	110
Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro	115	120	125
Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser	130	135	140
Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln	145	150	155
Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly	165	170	175
Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu	180	185	190
Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly	195	200	205
Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly	210	215	220
Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu	225	230	235
Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln	245	250	255
Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln			

**THIS PAGE BLANK (USPTO)**

260 265 270  
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe  
 275 280 285  
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met  
 290 295 300  
 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro  
 305 310 315 320  
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser  
 325 330 335  
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn  
 340 345 350  
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
 355 360 365  
 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp  
 370 375 380  
 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu  
 385 390 395 400  
 Gly Ala Ala

&lt;210&gt; 4

&lt;211&gt; 1288

&lt;212&gt; DNA

<213> *Erwinia amylovora*

&lt;400&gt; 4

aagcttcggc atggcacggt tgaccggttg gtcggcaggg tacgtttgaa ttattcataa 60  
 gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct 120  
 atcggcggtg cgggcgga aaacgggttg ctgggtacca gtcgccagaa tgctgggttg 180  
 ggtggcaatt ctgcaactgg gctgggcggc ggtaatcaaa atgataaccg caatcagctg 240  
 gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcggttg tgggctgatg 300  
 ggcgggtggc taggcggttg cttaggtaat ggcttgggtg gctcaggttg cctgggcgaa 360  
 ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa 420  
 ggcggcaaca ataccacttc aacaacaaat tccccgctgg accaggcgct ggggtattaac 480  
 tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac 540  
 ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tgggtgatgg 600  
 caagatggca ccagggcgag ttcctctggg ggcaagcagc cgaccgaagg cgagcagaac 660  
 gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag 720

**THIS PAGE BLANK (USPTO)**

```

ctccttgcca acgggggact gggaggtggt cagggcggtta atgctggcac gggctctgac 780
ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccggtgga ctaccagcag 840
ttaggtaacg ccgtgggtac cggtatcggt atgaaagcgg gcattcaggc gctgaatgat 900
atcggtacgc acaggcacag ttcaaccctg tctttcgtca ataaaggcga tcgggcgatg 960
gcgaaggaaa tcggtcagtt catggaccag tatectgagg tgtttggcaa gccgcagtac 1020
cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc 1080
aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc 1140
atgatcaaaa ggcccatggc ggtgatacc ggcaacggca acctgcaggc acgcggtgcc 1200
ggtggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca 1260
cttggcaagc tgggcgcggc ttaagctt 1288

```

&lt;210&gt; 5

&lt;211&gt; 447

&lt;212&gt; PRT

<213> *Erwinia amylovora*

&lt;400&gt; 5

```

Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
  1             5             10             15

```

```

Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
          20             25             30

```

```

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
      35             40             45

```

```

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
      50             55             60

```

```

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
      65             70             75             80

```

```

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
          85             90             95

```

```

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
      100             105             110

```

```

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
      115             120             125

```

```

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
      130             135             140

```

```

Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
      145             150             155             160

```

**THIS PAGE BLANK (USPTO)**

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser  
 165 170 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro  
 180 185 190

Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro  
 195 200 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro  
 210 215 220

Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly  
 225 230 235 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His  
 245 250 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln  
 260 265 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn  
 275 280 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val  
 290 295 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala  
 305 310 315 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr  
 325 330 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn  
 340 345 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp  
 355 360 365

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe  
 370 375 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser  
 385 390 395 400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser  
 405 410 415

**THIS PAGE BLANK (USPTO)**



Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu  
 420 425 430

Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu  
 435 440 445

<210> 6

<211> 1344

<212> DNA

<213> *Erwinia amylovora*

<400> 6

```

atgtcaattc ttacgcttaa caacaatacc tcgtcctcgc cgggtctgtt ccagtccggg 60
ggggacaacg ggcttggtgg tcataatgca aattctgcgt tggggcaaca acccatcgat 120
cggcaaacca ttgagcaaat ggctcaatta ttggcggaac tgttaaagtc actgctatcg 180
ccacaatcag gtaatgcggc aaccggagcc ggtggcaatg accagactac aggagttggt 240
aacgctggcg gcctgaacgg acgaaaaggc acagcaggaa ccaactccgca gtctgacagt 300
cagaacatgc tgagtgcgat gggcaacaac gggctggatc aggccatcac gcccgatggc 360
cagggcgggc ggcagatcgg cgataatcct ttactgaaag ccatgctgaa gcttattgca 420
cgcatgatgg acggccaaag cgatcagttt ggccaacctg gtacgggcaa caacagtgcc 480
tcttcoggta cttcttcacg tggcggttcc ccttttaacg atctatcagg ggggaaggcc 540
ccttcoggca actccccttc cggcaactac tctcccgta gtaccttctc acccccatcc 600
acgccaacgt cccctacctc accgcttgat ttcccttctt ctcccaccaa agcagccggg 660
ggcagcacgc cggtaaccga tcactcctgac cctgttggtg gcgcgggcat cggggccgga 720
aattcggttg ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc 780
gtgaaagcgg gtcaggtgtt tgatggcaaa ggacaaacct tcaccgccgg ttcagaatta 840
ggcgatggcg gccagtctga aaaccagaaa cgcgtgttta tactggaaga cggtgccagc 900
ctgaaaaacg tcaccatggg cgacgacggg gcggatggta ttcactctta cggtgatgcc 960
aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac 1020
agcgcgggca aaaaatccca cgttgaaatc actaacagtt ccttcgagca cgcctctgac 1080
aagatcctgc agctgaatgc cgatactaac ctgagcggtg acaacgtgaa ggccaaagac 1140
tttggtactt ttgtacgcac taacggcggt caacagggtg actgggatct gaatctgagc 1200
catatcagcg cagaagacgg taagttctcg ttcgttaaaa gcgatagcga ggggctaaac 1260
gtcaatacca gtgatatctc actgggtgat gttgaaaacc actacaaagt gccgatgtcc 1320
gccaacctga aggtggctga atga 1344

```

<210> 7

<211> 341

<212> PRT

<213> *Pseudomonas syringae*

<400> 7

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
 1 5 10 15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser

**THIS PAGE BLANK (USPTO)**

20	25	30
Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met		
35	40	45
Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala		
50	55	60
Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val		
65	70	75
Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe		
85	90	95
Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met		
100	105	110
Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu		
115	120	125
Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met		
130	135	140
Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro		
145	150	155
Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe		
165	170	175
Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile		
180	185	190
Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly		
195	200	205
Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser		
210	215	220
Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser		
225	230	235
Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp		
245	250	255
Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val		
260	265	270
Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln		

**THIS PAGE BLANK (USPTO)**

275                      280                      285  
 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
 290                      295                      300  
 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
 305                      310                      315                      320  
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
 325                      330                      335  
 Asn Gln Ala Ala Ala  
 340

&lt;210&gt; 8

&lt;211&gt; 1026

&lt;212&gt; DNA

<213> *Pseudomonas syringae*

&lt;400&gt; 8

atgcagagtc tcagtcttaa cagcagctcg ctgcaaacc cggcaatggc ccttgctctg 60  
 gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagttgtc 120  
 gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga 180  
 aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc 240  
 atcgtgcgc tggacaagct gatccatgaa aagctcgggtg acaacttcgg cgcgtctgcg 300  
 gacagcgcct cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc 360  
 aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac 420  
 gatatgccga tgctgaacaa gatcgcgag ttcatggatg acaatcccgc acagtttccc 480  
 aagccggact cgggctcctg ggtgaacgaa ctcaaggag acaacttcct tgatggcgac 540  
 gaaacggctg cgttccgttc ggactcgcac atcattggcc agcaactggg taatcagcag 600  
 agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcactccgag cagtttttcc 660  
 aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc 720  
 ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780  
 tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgagac cggtagctcg 840  
 gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggctt gctgctcaag 900  
 ggcttgagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960  
 gcgcaaactc ccaccttgct ggtcagtaac ctgctgcaag gcacccgcaa tcaggctgca 1020  
 gcctga 1026

&lt;210&gt; 9

&lt;211&gt; 424

&lt;212&gt; PRT

<213> *Pseudomonas syringae*

&lt;400&gt; 9

Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu

**THIS PAGE BLANK (USPTO)**

1	5	10	15
Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly	20	25	30
Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly	35	40	45
Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val	50	55	60
Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile	65	70	75
Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr	85	90	95
Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln	100	105	110
Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser	115	120	125
Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr	130	135	140
Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly	145	150	155
Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly	165	170	175
Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr	180	185	190
Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr	195	200	205
Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile	210	215	220
Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp	225	230	235
Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp	245	250	255
Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr			

**THIS PAGE BLANK (USPTO)**



12

**THIS PAGE BLANK (USPTO)**

```

cccgagtgca ctgttggtcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga 600
cagcaccgtc cagaatccgc aggacgccag caagcccaac gacagccagt ccaacatcgc 660
taaattgata agtgcattga tcatgtcggt gctgcagatg ctcaccaact ccaataaaaa 720
gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcgggct 780
cggtacaccg tcggccgata gcgggggcg cggtacaccg gatgcgacag gtggcggcgg 840
cggtgatacg ccaagcgcaa caggcggtgg cggcggtgat actccgaccg caacaggcgg 900
tggcggcagc ggtggcggcg gcacaccac tgcaacaggt ggcggcagcg gtggcacacc 960
cactgcaaca ggcggtggcg aggttggtg aacaccgcaa atcactccgc agttggccaa 1020
ccctaaccgt acctcaggta ctggctcggt gtcggacacc gcaggttcta ccgagcaagc 1080
cggcaagata aatgtggtga aagacaccat caaggctcggc gctggcgaag tctttgacgg 1140
ccacggcgca accttactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca 1200
gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa 1260
cgaggtcgat ggcattccacg tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt 1320
gcatgccagc aacgtcggtg aagacctgat tacggtcaaa ggcgagggag gcgcagcggt 1380
cactaatctg aacatcaaga acagcagtcg caaagggtgca gacgacaagg ttgtccagct 1440
caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggt 1500
tcgcaccaac ggtggcaagc agtttgatga catgagcatc gagctgaacg gcatcgaagc 1560
taaccacggc aagttcgccc tggtgaaaag cgacagtgc gatctgaagc tggcaacggg 1620
caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgaccaaca 1680
caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc 1729

```

&lt;210&gt; 11

&lt;211&gt; 344

&lt;212&gt; PRT

<213> *Pseudomonas solanacearum*

&lt;400&gt; 11

```

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
  1              5              10              15

```

```

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
      20              25              30

```

```

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
      35              40              45

```

```

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
      50              55              60

```

```

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
      65              70              75              80

```

```

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
      85              90              95

```

```

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
      100              105              110

```

**THIS PAGE BLANK (USPTO)**

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
 115 120 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
 130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
 145 150 155 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
 165 170 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
 180 185 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
 195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
 210 215 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
 225 230 235 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
 245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
 260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
 275 280 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
 290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
 305 310 315 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
 325 330 335

Gln Ser Thr Ser Thr Gln Pro Met  
 340

<210> 12

**THIS PAGE BLANK (USPTO)**

&lt;211&gt; 1035

&lt;212&gt; DNA

&lt;213&gt; Pseudomonas solanacearum

&lt;400&gt; 12

```
atgtcagtcg gaaacatcca gagcccgctc aacctcccgg gtctgcagaa cctgaacctc 60
aacaccaaca ccaacagcca gcaatcgggc cagtccgtgc aagacctgat caagcaggtc 120
gagaaggaca tcctcaacat catcgagcc ctcgtgcaga aggccgcaca gtcggcgggc 180
ggcaacaccg gtaacaccgg caacgcgccc gcgaaggacg gcaatgccaa cgcggggcgcc 240
aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc 300
ggcaacgtcg acgacgcca caaccaggat ccgatgcaag cgctgatgca gctgctggaa 360
gacctggtga agctgctgaa ggcggccctg cacatgcagc agcccggcgg caatgacaag 420
ggcaacggcg tgggcggtgc caacggcgcc aagggtgccc gcggccaggc cggcctggcc 480
gaagcgctgc aggagatcga gcagatcctc gccagctcg gcggcgggcg tgctggcgcc 540
ggcgggcgcg gtggcggtgt cggcggtgct ggtggcgcg atggcggtc cggcgcggt 600
ggcgcgaggc gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac 660
ggcccgcaga acgcaggcga tgtcaacggt gccaacggcg cggatgacgg cagcgaagac 720
cagggcgggc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctggtgcag 780
atgatgcagc aaggcggcct cggcgggcgg aaccaggcgc agggcggtc gaagggtgcc 840
ggcaacgcct cgccggcttc cggcgcgaa ccggcgcgga accagcccgg ttcggcggat 900
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc 960
gtccagatcc tgcagcagat gctggcgggc cagaacggcg gcagccagca gtccacctcg 1020
acgcagccga tgtaa 1035
```

**THIS PAGE BLANK (USPTO)**



**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau**INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)**

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C12N 15/82, C07K 14/195</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/20616</b> <b>(43) International Publication Date:</b> 13 April 2000 (13.04.00)
<b>(21) International Application Number:</b> PCT/US99/23265 <b>(22) International Filing Date:</b> 5 October 1999 (05.10.99) <b>(30) Priority Data:</b> 60/103,124 5 October 1998 (05.10.98) US <b>(71) Applicant:</b> EDEN BIOSCIENCE CORPORATION [US/US]; 11816 North Creek Parkway N., Bothell, WA 98011-8205 (US). <b>(72) Inventors:</b> WEI, Zhong-Min; 8230 125th Court, Kirkland, WA 98034 (US). SWANSON, Shane; 1027 N.E. 72nd Street, Seattle, WA 98115 (US). FAN, Hao; 19712 6th Drive S.E., Bothell, WA 98012 (US). <b>(74) Agents:</b> GOLDMAN, Michael, L. et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> HYPERSENSITIVE RESPONSE ELICITOR FROM <i>XANTHOMONAS CAMPESTRIS</i>  <b>(57) Abstract</b>  The present invention is directed to an isolated <i>Xanthomonas campestris</i> hypersensitive response elicitor protein or polypeptide. The hypersensitive response elicitor proteins or polypeptides in accordance with the present invention and the isolated DNA molecules that encode them have the following activities: imparting disease resistance to plants, enhancing plant growth, and/or to controlling insects on plants. This can be achieved by applying the hypersensitive response elicitor in a non-infectious form to plants or plant seed under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the elicitor can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

## HYPERSENSITIVE RESPONSE ELICITOR FROM *XANTHOMONAS CAMPESTRIS*

This application claims benefit of U.S. Provisional Patent Application  
5 Serial No. 60/103,124, filed October 5, 1998.

### FIELD OF THE INVENTION

The present invention relates to a *Xanthomonas campestris* from  
10 hypersensitive response elicitor.

### BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally  
15 fall into two categories: (1) compatible (pathogen-host), leading to intercellular  
bacterial growth, symptom development, and disease development in the host plant;  
and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a  
particular type of incompatible interaction occurring, without progressive disease  
symptoms. During compatible interactions on host plants, bacterial populations  
20 increase dramatically and progressive symptoms occur. During incompatible  
interactions, bacterial populations do not increase, and progressive symptoms do not  
occur.

The hypersensitive response is a rapid, localized necrosis that is  
associated with the active defense of plants against many pathogens (Kiraly, Z.,  
25 "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant  
Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed.  
Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177  
in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic  
Press, New York (1982)). The hypersensitive response elicited by bacteria is readily  
30 observed as a tissue collapse if high concentrations ( $\geq 10^7$  cells/ml) of a limited  
host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated  
into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower  
levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of  
Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

- 2 -

"Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangel, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "*Pseudomonas Syringae* pv. *Syringae* HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), Wei,

Z.-M., et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora*, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum* *popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "Erwinia chrysanthemi Harpin<sub>Ech</sub>: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA<sup>-</sup> Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention identifies yet another hypersensitive response elicitor protein or polypeptide.

## SUMMARY OF THE INVENTION

5 The present invention is directed to an isolated *Xanthomonas campestris* hypersensitive response elicitor protein or polypeptide.

The hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth, and/or controlling insects. This involves applying the hypersensitive response elicitor in a non-  
10 infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the hypersensitive response elicitor to plants or plant seeds in order to impart disease resistance, to enhance plant growth,  
15 and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a *Xanthomonas campestris* hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease  
20 resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a hypersensitive response elicitor can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or  
25 plants grown from the plant seeds.

## DETAILED DESCRIPTION OF THE INVENTION

30 The present invention is directed to an isolated *Xanthomonas campestris* hypersensitive response elicitor protein or polypeptide.

The hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth and/or controlling

- 5 -

insects. This involves applying the hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

5 As an alternative to applying the hypersensitive response elicitor to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a *Xanthomonas campestris*  
10 hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a hypersensitive response elicitor can be provided  
15 and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

The hypersensitive response elicitor polypeptide or protein derived from *Xanthomonas campestris* has an amino acid sequence corresponding to SEQ.  
20 ID. No. 1 as follows:

Met	Asp	Gly	Ile	Gly	Asn	His	Phe	Ser	Asn
1				5					10

25 This hypersensitive response elicitor polypeptide or protein has a molecular weight of 13-15 kDa.

Fragments of the above hypersensitive response elicitor polypeptides or proteins are encompassed by the present invention.

30 Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

- 6 -

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active  
5 elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular  
10 portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and  
15 pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated  
20 to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The hypersensitive response elicitor of the present invention is  
25 preferably in isolated form (i.e. separated from its host *Xanthomonas campestris*) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the hypersensitive response elicitor of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the  
30 present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein hypersensitive response elicitor, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical



- 7 -

treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor is separated by centrifugation. The supernatant fraction containing the hypersensitive response elicitor is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction  
5 may be further purified by ion exchange or HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an  
10 expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

15 U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic  
20 cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral  
25 vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W.  
30 Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced

- 8 -

into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct

- 9 -

positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P<sub>R</sub> and P<sub>L</sub> promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-

- 10 -

ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the hypersensitive response elicitor polypeptide or protein of the present invention, in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the elicitor to impart disease resistance, impart stress resistance, enhance growth, and/or control insects. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to impart stress resistance, to enhance plant growth, and/or to effect insect control.

As an alternative to applying the hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding the hypersensitive response elicitor polypeptide or protein, which fragment elicits a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding the hypersensitive response elicitor polypeptide or protein of the present invention can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of the isolated hypersensitive response elicitor or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seeds cells. In these  
10 embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly,  
15 useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato,  
20 sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute  
25 immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and  
30 avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of

- 12 -

pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention:

5 *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the hypersensitive response elicitor protein or  
10 polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased  
15 quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved  
20 crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the  
25 hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease  
30 damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on

- 13 -

over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

The hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the elicitor can be applied separately to plants with other materials being applied at different times.

- 14 -

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains the hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition  
5 contains greater than 500 nM of the elicitor.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

10 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

15 In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, the hypersensitive response elicitor of the present invention need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding the elicitor are produced according to procedures well known in the art.

20 The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby  
25 incorporated by reference.

Another approach to transforming plant cells with a gene is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent  
30 Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the



- 15 -

outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette.

Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

*Agrobacterium* is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

- 16 -

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants. Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively,

- 17 -

transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which the hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including the hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor in accordance with the present invention to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

## EXAMPLES

### Example 1 - Culture Growth

For the purpose of this study, *Xanthomonas campestris pelargonii* (*Xcp*) was grown on Luria agar plates. From these plates, colonies were transferred to inoculate *Xcp* seed cultures. The seed cultures were grown in 250ml baffled flasks containing 50ml of 50% Luria broth. The seed cultures were grown at approximately 27°C, while shaking at 250rpm, until an optical density ( $\lambda_{620}$ ) of 0.5 to 0.8 was reached.

To inoculate the minimal media, the seed cultures were centrifuged in sterile 500ml centrifuge bottles for 10 minutes at 4°C and 10,000 rpm. The

- 18 -

supernatant was discarded and the resulting cell pellet was resuspended in the already prepared minimal media, in a manner such that no Luria broth was introduced into the minimal media culture. A 1:10 ratio of seed culture to minimal media was consistently used for inoculating the minimal media. In other words, the cell pellet formed a 50ml seed culture which was used to inoculate 500ml of minimal media. The minimal media culture was grown in a 2.8L Fernbach flask containing 500ml of media at approximately 27°C which was agitated at 250 rpm until an optical density ( $\lambda 620$ ) of 1.7 to 2.0 was reached.

After the flask production of culture had been optimized, fermentation was transferred to 10L MicroFerm fermenter (New Brunswick Scientific, Edison, New Jersey, USA). For the 10L fermentation, the seed culture to minimal media ratio, as described above, was maintained. The fermentation was run at approximately 27°C with an initial pH of 6.0 and final pH of 5.8. The vessel was agitated at 400 rpm with 0.8 to 1.0 vessel volumes of air per minute.

A 1L 10x stock solution of the minimal media contained 39.2g of  $K_2HPO_4$ , 71.5g  $KH_2PO_4$ , 10.0g of  $(NH_4)_2SO_4$ , 3.5g of  $MgCl_2$ , 1.0g of NaCl, and 34.23g of sucrose with a final pH of 6.0 to 6.2 was prepared after thoroughly mixing. The stock was sterile filtered and kept at 4°C.

## **Example 2 – Cell-Free Elicitor Preparation**

The first step in the purification of the *Xcp* hypersensitive response elicitor was the development of a cell-free elicitor preparation ("CFEP"). The CFEP production involves four steps as described below.

### **1. Initial Centrifugation**

In the initial centrifugation step, the minimal media cell culture was divided into 370ml aliquots and centrifuged in 500ml centrifuge bottles for 10 minutes, at 4°C, and at 8,000 rpm. The resulting cell pellet was resuspended at a 1:10 weight to volume ratio using lysis buffer. The lysis buffer consisted of 10mM NaCl and 20mM Tris-HCL at pH 8.0. Resuspension of the cell pellet was achieved

- 19 -

by vortexing individual centrifuge bottles. In the case of the 10L fermentation, a homogenizer was used.

## 2. Sonication

5 The resuspended pellet was then sonicated in 50ml aliquots at a setting of 5 for 3 minutes (with the horn tip, VirTris, VirSonic). While sonicating, the beaker containing the solution was immersed in an ice water bath. The resulting sonicate was kept on ice until all of the solution was sonicated. The setting used for sonication  
10 was the manufacturer's suggested maximum setting for the tip used.

## 3. Heat Treatment

The sonicate was placed on a preheated stir plate and brought to a  
15 rolling boil. The rolling boil was sustained for 5 minutes. After the 5 minute heat treatment, the solution was immediately placed in an ice water bath and cooled to approximately 10°C.

## 4. Final Centrifugation

20 Prior to final centrifuging, the cooled solution was brought back to its original volume with deionized H<sub>2</sub>O, replacing the volume lost to evaporation during boiling. The solution was then centrifuged in 50ml centrifuge bottles for 30 minutes at 4°C and 15,000 rpm. The resulting supernatant from each bottle was combined and  
25 frozen in a -80°C freezer. A 1ml sample was also saved and used for testing hypersensitive response activity.

### Example 3 - Protein Verification

30 To determine whether the hypersensitive response elicitor was indeed a protein, protease digestions were performed with CFEP. CFEP, prepared as previously described, was inoculated with protease K at a concentration of 2mg/ml. After a 1.5h incubation at 37°C, the protease inoculated CFEP along with the positive control (CFEP alone) and negative control (protease K at 2mg/ml in lysis buffer) were  
35 infiltrated into tobacco plants for hypersensitive response elicitor testing. The

- 20 -

positive control showed hypersensitive response necrosis, while the negative control showed no signs of testing. The protease K inoculated CFEP also showed no signs of hypersensitive response, indicating the *Xcp* hypersensitive response elicitor was sensitive to protease digestion and indeed a protein. This experiment was repeated several times with different batches of CFEP, each time with the same results.

#### **Example 4 - Chromatographic Purification**

The production of a hypersensitive response elicitor CFEP was the first step in the purification scheme for the *Xcp* hypersensitive response elicitor. Further purification of the elicitor consisted of four chromatographic steps. Anion exchange, cation exchange, various types of affinity chromatography, hydrophobic interaction, and reversed phase chromatography media were all analyzed for their utility in purifying the hypersensitive response elicitor. All chromatography experiments were conducted with the FPLC and FPLC detector (Pharmacia Biotech, Uppsala, Sweden). The final purification scheme used consisted primarily of chromatography media that binded to the *Xcp* hypersensitive response elicitor based the hypersensitive response elicitor's hydrophobic characteristics.

##### **1. Butyl Sepharose**

The CFEP was first bound to a medium strength hydrophobic interaction chromatography medium. The CFEP was adjusted to 600mM NaCl and loaded on to a Butyl Sepharose 4 Fast Flow (Pharmacia Biotech, Uppsala, Sweden). The column was eluted with a 75-100% buffer B gradient. Buffer A contained 600mM NaCl, 20mM Tris-HCl at pH 8, and buffer B contained 10mM Tris-HCl at pH 8. At 85% B gradient, buffer B was exchanged for deionized H<sub>2</sub>O.

##### **2. Mono S**

The fractions judged to have the highest concentration of the hypersensitive response elicitor (determined by making hypersensitive response dilution series with active fractions) from the Butyl Sepharose column were pooled together and loaded on to a strong cation exchanger, Mono S (Mono S 10/10 column,

- 21 -

Pharmacia Biotech). Prior to loading, the pooled fractions were adjusted to 20mM NaCl, 20mM Tris-HCl at pH 5.5. Buffer A was 20mM NaCl, 20mM Tris-HCl at pH 5.5. Buffer B contained 1M NaCl, 20mM Tris-HCl at pH 5.5. The sample was loaded, followed by a 0% buffer B wash and then 100% buffer B wash. The hypersensitive response elicitor did not bind to the Mono S medium, but, at pH 5.5, many of the contaminants in the sample did. Thus, it served as a non-binding chromatography. Immediately following the collection of the flow through (the hypersensitive response elicitor fraction), the solution's pH was adjusted to 8.0.

### 3. Phenyl Sepharose (low)

The active fraction from the Mono S column was then loaded on to a Phenyl Sepharose 6 Fast Flow low substitution (a weak hydrophobic interaction medium, Pharmacia Biotech, Uppsala, Sweden). Buffer A contained 1M NaCl, 20mM Tris-HCl at pH 8, and buffer B contained 10mM Tris-HCl at pH 8.0. As mentioned previously, at 85% B, buffer B was changed to deionized H<sub>2</sub>O. The hypersensitive response elicitor fraction was eluted as the gradient reached 100%B. The fractions which were then used in the next purification step contained the highest concentration of the hypersensitive response elicitor. Since the active fraction eluted in deionized H<sub>2</sub>O, it was necessary to stabilize the fraction by adjusting them to 20mM NaCl and 20mM Tris-HCl at pH 8.0.

### 4. Reversed Phase Chromatography

The final chromatographic step in the purification of the hypersensitive response elicitor utilized a ProRPC 5/5, reversed phase column (Pharmacia Biotech, Uppsala, Sweden). The sample was adjusted to 15% acetonitrile (HPLC grade) and 0.1% TFA (HPLC grade) and loaded at a flow rate of 0.7ml/min. After a 5ml wash, a 15-50% B gradient was run over 58ml at a flow rate of 0.7ml/min. 1ml fractions were collected for the entire volume of the gradient. The *Xcp* hypersensitive response elicitor eluted at approximately 25%B. Fractions containing the hypersensitive response elicitor were determined by visual identification on a silver stained PAGE-SDS gels. After visual identification, fractions that were relatively pure and contained high concentrations of the elicitor were pooled and dialyzed against the previously

- 22 -

mentioned lysis buffer to remove the acetonitrile and TFA. The dialyzed fraction was then concentrated approximately 100 fold using a Centricon 3.000 MWCO (Amicon, Beverly, MA).

5 **Example 5 - Electroelution of the *Xcp* Hypersensitive Response Elicitor**

The final concentrate was then run on a hand poured 18% acrylimide PAGE-SDS mini gel (gel was poured in an empty Novex gel cassette and run with a Novex X-Cell apparatus, San Diego, CA). The gel was stained and destained with  
10 normal Coomassie Blue staining techniques. Because of extreme overloading of the gel, it was possible to see and cut out the band that correlated with the hypersensitive response elicitor. The cut out gel was then loaded into an Elutrap (Schleicher & Schuell, Keene, New Hampshire). The protein present on the cut out was eluted off the gel, and into a collection chamber. A non-SDS tank buffer was used in the  
15 Elutrap to produce a sample relatively free of SDS. A portion of the resulting fraction was then run on a 16% acrylimide PAGE-SDS mini gel (Novex gel and apparatus). The gel was silver stained in order to determine the sample's purity. In addition, a portion of the eluted fraction was used to make hypersensitive response elicitor dilutions to determine the relative concentration of hypersensitive response elicitor  
20 present in the final sample.

**Example 6 - Visualization of the Hypersensitive Response Elicitor**

Perhaps the most significant and troublesome characteristic  
25 encountered during the isolation of the *Xcp* hypersensitive response elicitor was its abnormal staining characteristics. PAGE-SDS gels that had been stained and destained using normal Coomassie Blue techniques showed the hypersensitive response elicitor band only when the gel was extremely overloaded. Even under overloaded conditions, the elicitor stained very faintly and to some extent temporarily.  
30 Complete destaining of the gel usually resulted in fading of the hypersensitive response elicitor band. Only with silver staining techniques was it possible to visualize the elicitor at a seemingly lower concentration. The band, depending of the concentration of the hypersensitive response elicitor present in the sample, either



- 23 -

appeared as a negatively stained band with relatively distinct borders (at higher concentration) or as a slightly off-colored band, almost resembling a shadow with non-distinct, fuzzy borders. Silver stained PAGE-SDS gels were used to visualize the hypersensitive response elicitor in all cases, except when the gel was to be used for electroelution with the Elutrap or protein transfer on to a PVDF membrane.

As in the case of Coomassie Blue staining of PAGE-SDS gels, Coomassie Blue staining of PVDF membranes (used to immobilize the protein for N-terminal sequencing) resulted in very faint staining if intensely overloaded. Normal loading volumes resulted in a seemingly clean membrane with no apparent protein bands.

#### **Example 7 - N-terminal Sequence of the *Xcp* Hypersensitive Response Elicitor**

After purification of the elicitor had been achieved it was then possible to learn the N-terminal sequence of the protein. The purified *Xcp* hypersensitive response elicitor protein was run on a SDS-PAGE. From this gel, the protein was transferred to a PVDF membrane via a Trans-Blot (Bio-Rad, Hercules, CA). The membrane containing the protein was subjected to an automated Edmans Degradation process followed by high-pressure liquid chromatography for detection and identification of the individual amino acids. The first ten amino acids of the *Xcp* hypersensitive response elicitor were determined to be:

Met	Asp	Gly	Ile	Gly	Asn	His	Phe	Ser	Asn
1				5					10

(SEQ. ID. No. 1).

#### **Example 8 - Elicitor-Induced Disease Resistance in Tobacco and Tomato**

The hypersensitive response elicitor of *Xanthomonas campestris* pv. *pelargonii* was sprayed on tobacco plants at a concentration of approximately 5 ppm. Three days after the treatment, the plants were inoculated with tobacco mosaic virus (TMV) at a concentration of ca. 2 ppm. Four days after inoculation, the elicitor treated plants showed more than a 65% reduction in the number of lesions compared

- 24 -

to untreated control plants. In addition to resistance to TMV, the hypersensitive response elicitor also induced resistance to bacterial wilt of tomato.

**Example 9 - The Hypersensitive Response Elicitor-Induced Growth Enhancement of Tomato**

5

To demonstrate that the hypersensitive response elicitor of *Xanthomonas campestris pv. pelargonii* can enhance plant growth, tomato seeds were soaked in an elicitor solution of ca. 5 ppm for more than 4 hours. The seeds soaked in the same solution but without the elicitor protein were used as untreated control. Both the treated and untreated seeds were planted in eight inch pots with artificial soil, 20 seeds per pot. Twenty days after planting, the size of the tomato seedlings were measured. The plants derived from the elicitor treated seeds were 10% fuller than those generated from the untreated seeds.

15

A proteinaceous hypersensitive response elicitor has been purified to near homogeneity from the plant pathogen *Xanthomonas campestris pelargonii*. The protein has a molecular weight of approximately 14kDa and is heat tolerant. The hypersensitive response elicitor exhibits unusual staining characteristics when stained with normal Coomassie Blue and silver staining techniques. The *Xanthomonas campestris pelargonii* hypersensitive response elicitor is a member of the hypersensitive response elicitor protein family due to its shared characteristics with other proteins of this family. These characteristics include biochemical characteristics, the ability to elicit a hypersensitive response, and, from preliminary experiments, the ability to induce disease resistance and plant growth enhancement.

20

Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

25

30

- 25 -

**WHAT IS CLAIMED:**

1. An isolated *Xanthomonas campestris* hypersensitive response elicitor protein or polypeptide.  
5
2. An isolated hypersensitive response elicitor protein or polypeptide according to claim 1, wherein the protein or polypeptide has a molecular weight of 13-15 kDa.
- 10 3. An isolated hypersensitive response elicitor protein or polypeptide according to claim 1, wherein the protein or polypeptide has an amino acid sequence of SEQ. ID. No. 1.
- 15 4. A method of imparting disease resistance to plants comprising:  
applying a hypersensitive response elicitor protein or polypeptide according to claim 1 in a non-infectious form to a plant or plant seed under conditions effective to impart disease resistance.
- 20 5. A method according to claim 4, wherein plants are treated during said applying.
- 25 6. A method according to claim 4, wherein plant seeds are treated during said applying, said method further comprising:  
planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.
- 30 7. A method of enhancing plant growth comprising:  
applying a hypersensitive response elicitor protein or polypeptide according to claim 1 in a non-infectious form to a plant or plant seed under conditions effective to enhance plant growth.

- 26 -

8. A method according to claim 7, wherein plants are treated during said applying.

5 9. A method according to claim 7, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

10 10. A method of insect control for plants comprising:  
applying hypersensitive response elicitor protein or polypeptide according to claim 1 in a non-infectious form to a plant or plant seed under conditions effective to control insects.

15 11. A method according to claim 10, wherein plants are treated during said applying.

12. A method according to claim 10, wherein plant seeds are treated during said applying, said method further comprising:

20 planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

## SEQUENCE LISTING

<110> Eden Bioscience Corporation

<120> HYPERSENSITIVE RESPONSE ELICITOR FROM XANTHOMONAS  
CAMPESTRIS

<130> 21829/52

<140>

<141>

<150> 60/103,124

<151> 1998-10-05

<160> 1

<170> PatentIn Ver. 2.0

<210> 1

<211> 10

<212> PRT

<213> Xanthomonas campestris

<400> 1

Met Asp Gly Ile Gly Asn His Phe Ser Asn  
1 5 10

**THIS PAGE BLANK (USPTO)**

# INTERNATIONAL SEARCH REPORT

Inter. Application No

PCT/US 99/23265

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C12N15/82 C07K14/195

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SWANSON, S. ET AL.: "Isolation and characterization of an HR elicitor from Xanthomonas campestris." PHYTOPATHOLOGY, vol. 88, no. 9 SUPPL., September 1998 (1998-09), page S87 XP000866309	1-3
Y	the whole document	4-12
X	WO 98 24297 A (CORNELL RES FOUNDATION INC) 11 June 1998 (1998-06-11)	1
Y	page 12, line 16 -page 13, line 25 page 25, line 39 -page 26, line 16 abstract	4-6
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

13 January 2000

Date of mailing of the international search report

20/01/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040. Tx. 31 651 epo nl.  
Fax: (+31-70) 340-3016

Authorized officer

Mata Vicente, T.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/23265

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 32844 A (CORNELL RES FOUNDATION INC) 30 July 1998 (1998-07-30)	1
Y	page 9, line 3 -page 10, line 7 page 22, line 9 - line 37 abstract	7-9
X	WO 98 37752 A (CORNELL RES FOUNDATION INC) 3 September 1998 (1998-09-03)	1
Y	page 3, line 27 -page 4, line 29 page 16, line 9 - line 37	10-12



# INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr. 1st Application No

PCT/US 99/23265

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9824297 A	11-06-1998	AU 5693598 A EP 0957672 A	29-06-1998 24-11-1999
WO 9832844 A	30-07-1998	AU 6043198 A	18-08-1998
WO 9837752 A	03-09-1998	AU 6666498 A	18-09-1998

**THIS PAGE BLANK (USPTO)**

09/880-371

13

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A01N 63/02</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/11133</b> <b>(43) International Publication Date:</b> 11 March 1999 (11.03.99)
<b>(21) International Application Number:</b> PCT/US98/17252 <b>(22) International Filing Date:</b> 20 August 1998 (20.08.98)  <b>(30) Priority Data:</b> 60/057,464 3 September 1997 (03.09.97) US  <b>(71) Applicants:</b> CORNELL RESEARCH FOUNDATION, INC. [US/US]; Suite 105, 20 Thornwood Drive, Ithaca, NY 14850 (US). EDEN BIOSCIENCE CORPORATION [GB/GB]; 11816 North Creek Parkway N., Bothell, WA 98011-8205 (GB).  <b>(72) Inventors:</b> BEER, Steven, V.; 211 Hudson Street, Ithaca, NY 14850 (US). BUTLER, Jerry, L.; 15100 176th Avenue N.E., Woodinville, WA 98072 (US).  <b>(74) Agents:</b> GOLDMAN, Michael, L. et al.; Nixon, Hargrave, Devans & Doyle LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> USE OF HYPERSENSITIVE RESPONSE ELICITOR FROM GRAM POSITIVE BACTERIA  <b>(57) Abstract</b>  The present invention is directed to the use of a protein or polypeptide from Gram positive bacteria, such as <i>Clavibacter michiganensis</i> subsp. <i>sepedonicus</i> , which elicits a hypersensitive response in plants. This protein or polypeptide can be used to impart disease resistance to plants, to enhance plant growth, and/or to control insects on plants. This can be achieved by applying the hypersensitive response elicitor protein or polypeptide in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

## USE OF HYPERSENSITIVE RESPONSE ELICITOR FROM GRAM POSITIVE BACTERIA

5

### FIELD OF THE INVENTION

The present invention relates to the use of the hypersensitive response elicitor from Gram positive bacteria, such as *Clavibacter michiganensis* subsp. *sepedonicus*, for disease resistance, growth enhancement, and insect control.

This application claims benefit of U.S. Provisional Patent Application Serial No. 60/057,464, filed September 3, 1997.

### BACKGROUND OF THE INVENTION

15

Interactions between bacterial pathogens and their plant hosts generally fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.

25

The hypersensitive response ("HR") is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z., "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ( $\geq 10^7$  cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower

30

- 2 -

levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al., "Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation  
5 Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z.,  
10 "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene  
15 Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.  
20 The *hrp* genes are widespread in gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and  
25 Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends  
30 Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "Pseudomonas

- 3 -

*Syringae* pv. *Syringae* Harpin<sub>PSS</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), Wei, Z.-H., et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al.

- 5 "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei,  
10 Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical  
15 properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum* *popA* mutants still elicit the hypersensitive response in tobacco and  
20 incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "Erwinia chrysanthemi Harpin<sub>Ech</sub>: Soft-Rot Pathogenesis," MPMI 8(4): 484-91  
25 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA<sup>+</sup> Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and  
30 Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

- 4 -

The present invention is directed to the use of a hypersensitive response elicitor protein or polypeptide from Gram positive bacteria.

## SUMMARY OF THE INVENTION

5

The hypersensitive response eliciting protein or polypeptide from Gram positive bacteria, such as *Clavibacter*, particularly *Clavibacter michiganensis* subsp. *sepedonicus*, can be used to impart disease resistance to plants, to enhance plant growth, and/or to control insects. This involves applying the hypersensitive response elicitor protein or polypeptide in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the gram positive hypersensitive response elicitor protein or polypeptide to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding the hypersensitive response gram positive elicitor protein or polypeptide and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds.

Alternatively, a transgenic plant seed transformed with the DNA molecule encoding the gram positive hypersensitive response elicitor protein or polypeptide can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of the hypersensitive response elicitor protein or polypeptide from Gram positive bacteria, such as a *Clavibacter*, particularly *Clavibacter michiganensis* subsp. *sepedonicus*, for disease resistance, growth enhancement, and insect control in plants. This hypersensitive response



- 5 -

elicitor protein or polypeptide is described in and can be isolated according to Nissinen, et al., "*Clavibacter michiganensis* subsp. *sepedonicus* Elicits a Hypersensitive Response in Tobacco and Secretes Hypersensitive Response-Inducing Protein(s)," Phytopathology, pp. 678-84 (1997), which is hereby incorporated by  
5 reference.

The gene encoding this hypersensitive response elicitor protein or polypeptide can be obtained by procedures well known in the art. The protein can be purified through conventional techniques such as chromatography or electrophoresis. The amino terminal sequence of the protein is determined and used to design  
10 degenerate oligonucleotides which are labelled and used as probes to screen a clone library. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), which is hereby incorporated by reference. Plasmid DNA is isolated and sequenced from clones which hybridize to the amino terminal probe. The sequence of the DNA molecule can be determined  
15 using either chemical (Maxam et al., Proc. Natl. Acad. Sci. USA, 74:560 (1977), which is hereby incorporated by reference ) or enzymatic (Sanger, et al., Proc. Natl. Acad. Sci. USA, 74:5463 (1977), which is hereby incorporated by reference) methods.

Fragments of the above hypersensitive response elicitor polypeptide or  
20 protein are encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding the elicitor protein of the present invention are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to  
25 yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely  
30 to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

- 6 -

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for increased expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The protein or polypeptide of the present invention is preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, differential pressure, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the polypeptide or protein of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant

- 7 -

DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria

- 8 -

transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression  
5 elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).  
10 Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a  
15 procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called  
20 the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct  
25 positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use  
30 strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its

- 9 -

bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P<sub>R</sub> and P<sub>L</sub> promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

- 10 -

The present invention relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions where the polypeptide or protein contacts all or part of the cells of the plant or plant seed. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor polypeptide or protein; 2) application of bacteria which do not cause disease and are transformed with genes encoding a hypersensitive response elicitor polypeptide or protein; and 3) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and naturally contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

In one embodiment of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be isolated from its bacterial source.

In other embodiments of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be applied to plants or plant seeds by applying bacteria containing genes encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the polypeptide or protein so that the elicitor can contact plant or plant seed cells. In these embodiments, the hypersensitive response elicitor polypeptide or protein is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

In one embodiment of the bacterial application mode of the present invention, the bacteria do not cause the disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive response elicitor polypeptide or protein. For example, *E. coli*, which does not elicit a hypersensitive response in plants, can be transformed with genes encoding a hypersensitive response elicitor polypeptide or protein and then applied to plants. Bacterial species other than *E. coli* can also be used in this embodiment of the present invention.

In another embodiment of the bacterial application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a hypersensitive response elicitor polypeptide or protein. Examples of such bacteria are noted above. However, in this embodiment, these bacteria are applied to plants or their seeds which are not susceptible to the disease carried by the bacteria.

The method of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are:

- 12 -

*Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention: *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved



- 13 -

crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, and tomato pinworm. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include topical applications, such as high or low pressure spraying, as well as injection and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds, in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by topical procedures, such as low or high pressure spraying, coating, or immersion, as well as by injection. Other suitable application

- 14 -

procedures can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

10           The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials being applied at different times.

15           A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM hypersensitive response elicitor polypeptide or protein.

20           Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

25           Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

30           In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive

- 15 -

response elicitor polypeptide or protein are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA.

- 5 Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

- 10 Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure
- 15 involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is
- 20 carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

- Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies.
- 25 Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

- The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are
- 30 electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes

- 16 -

allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing

- 17 -

transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

10           After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

          Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively, transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

20           When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to impart

- 18 -

disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

Although the invention has been described in detail for the purpose of illustration, it is understood that such details are solely for that purpose and that  
5 variations can be made therein by those skilled in the art without departing from the spirit of the scope of the invention which is defined by the following claims.

## WHAT IS CLAIMED:

1. A method of imparting disease resistance to plants comprising:  
applying a hypersensitive response elicitor protein or  
5 polypeptide from a Gram positive bacterium, in a non-infectious form, to a plant or  
plant seed under conditions where the protein or polypeptide contacts the cells of the  
plants or the plant seeds and imparts disease resistance.
2. A method according to claim 1, wherein plants are treated  
10 during said applying.
3. A method according to claim 1, wherein plant seeds are treated  
during said applying, said method further comprising:  
planting the seeds treated with the hypersensitive response  
15 elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.
4. A method according to claim 1, wherein the Gram positive  
bacterium is a *Clavibacter*.
- 20 5. A method according to claim 4, wherein the *Clavibacter* is  
*Clavibacter michiganensis* subsp. *sepedonicus*.
6. A method of enhancing plant growth comprising:  
25 applying a hypersensitive response elicitor protein or  
polypeptide from a Gram positive bacterium, in a non-infectious form, to a plant or  
plant seed under conditions where the protein or polypeptide contacts the cells of the  
plants or the plant seeds and enhances plant growth.
- 30 7. A method according to claim 6, wherein plants are treated  
during said applying.

- 20 -

8. A method according to claim 6, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and  
5 propagating plants from the seeds planted in the soil.

9. A method according to claim 6, wherein the Gram positive bacterium is a *Clavibacter*.

10. A method according to claim 9 wherein the *Clavibacter* is *Clavibacter michiganensis* subsp. *sepedonicus*.

11. A method of insect control for plants comprising:  
applying a hypersensitive response elicitor protein or  
15 polypeptide from a Gram positive bacterium, in a non-infectious form, to a plant or plant seed under conditions where the protein or polypeptide contacts the cells of the plants or the plant seeds and controls insects.

12. A method according to claim 11, wherein plants are treated  
20 during said applying.

13. A method according to claim 11, wherein plant seeds are treated during said applying, said method further comprising:  
planting the seeds treated with the hypersensitive response  
25 elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.

14. A method according to claim 11, wherein the Gram positive bacterium is a *Clavibacter*.

15. A method according to claim 14, wherein the *Clavibacter* is *Clavibacter michiganensis* subsp. *sepedonicus*.



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/17252

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A01N63/02

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88 09114 A (CROP GENETICS INTERNATIONAL) 1 December 1988 see page 4, line 1 - line 111 see page 6, line 1 - line 12 see page 6, line 28 - page 8, line 5	1-15
X	WO 90 13224 A (CROP GENETICS INTERNATIONAL) 15 November 1990 see page 3, line 26 - page 4, line 14 see page 6, line 25 - line 36 see examples 1-11	1-15
X	WO 91 10363 A (CROP GENETICS INTERNATIONAL) 25 July 1991 see the whole document	1-15
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

4 December 1998

Date of mailing of the international search report

21/12/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Fort, M

# INTERNATIONAL SEARCH REPORT

Inter. Appl. No.  
PCT/US 98/17252

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 589 110 A (PLANT GENETIC SYSTEMS) 30 March 1994 see page 2, line 5 - line 8 see page 3, line 39 - line 54 ---	1-15
X	CHEMICAL ABSTRACTS, vol. 120, no. 15, 11 April 1994 Columbus, Ohio, US; abstract no. 184202, LAMPEL, JAY S. ET AL: "Integrative cloning, expression, and stability of the cryIA(c) gene from Bacillus thuringiensis subsp. kurstaki in a recombinant strain of Clavibacter xyli subsp. cynodontis" XP002086718 see abstract & APPL. ENVIRON. MICROBIOL. (1994), 60(2), 501-8 CODEN: AEMIDF; ISSN: 0099-2240, ---	1-15
X	CHEMICAL ABSTRACTS, vol. 127, no. 4, 28 July 1997 Columbus, Ohio, US; abstract no. 47639, METZLER, MARY C. ET AL: "The status of molecular biological research on the plant pathogenic genus Clavibacter" XP002086719 see abstract & FEMS MICROBIOL. LETT. (1997), 150(1), 1-8 CODEN: FMLED7; ISSN: 0378-1097, ---	1-15
X	BIOLOGICAL ABSTRACTS, vol. 95, Philadelphia, PA, US; abstract no. 452043, TOMASINO S F ET AL: "Field performance of Clavibacter xyli subsp. cynodontis expressing the insecticidal protein gene cryIA(c) of Bacillus thuringiensis against European corn borer in field corn." XP002086716 see abstract & BIOLOGICAL CONTROL 5 (3). 1995. 442-448. ISSN: 1049-9644, ---	1-15
	-/-	

# INTERNATIONAL SEARCH REPORT

Inter. Appl. No.

PCT/US 98/17252

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>BIOLOGICAL ABSTRACTS, vol. 95, Philadelphia, PA, US; abstract no. 350265, NISSINEN R ET AL: "Clavibacter michiganensis subsp. sepedonicus elicits a hypersensitive response in tobacco and secretes hypersensitive response-inducing protein (s)." XP002086717 see abstract &amp; PHYTOPATHOLOGY 87 (7). 1997. 678-684. ISSN: 0031-949X, cited in the application -----</p>	1-15

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/US 98/17252

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8809114 A	01-12-1988	AT 112442 T AU 620810 B AU 1807588 A CA 1320352 A DE 3851756 D DE 3851756 T EP 0358718 A JP 3501800 T US 5415672 A	15-10-1994 27-02-1992 21-12-1988 20-07-1993 10-11-1994 02-02-1995 21-03-1990 25-04-1991 16-05-1995
WO 9013224 A	15-11-1990	AU 5553190 A CA 2053295 A EP 0470182 A JP 4504722 T	29-11-1990 29-10-1990 12-02-1992 20-08-1992
WO 9110363 A	25-07-1991	AU 7159291 A	05-08-1991
EP 0589110 A	30-03-1994	US 5628995 A US 5530197 A	13-05-1997 25-06-1996